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CONTENTS

No. 1, JANUARY 25, 1906.

PAGE

I. FLEXNER, SIMON, and NOGUCHI, HIDEYO. The Effect of Eosin upon Tetanus Toxin and upon Tetanus in Rats and Guinea-Pigs 1

II. ERLANGER, JOSEPH. On the Physiology of Heart-Block in Mammals, with Especial Reference to the Causation of Stokes-Adams Disease 8

Plates I–IV.

III. BURTON-OPITZ, R. The Effect of Changes in Temperature upon the Viscosity of the "Living" Blood 59

IV. PEARCE, RICHARD M. Experimental Cirrhosis of the Liver 64

Plate V.

V. PEARCE, RICHARD M., and STANTON, E. MACD. Experimental Arteriosclerosis 74

Plates VI and VII.

VI. NOGUCHI, HIDEYO. On Certain Thermostabile Venom Activators 87

VII. OERTEL, HORST. A Further Contribution to the Knowledge of Multiple Non-Inflammatory Necrosis of the Liver with Jaundice (Hepar Necroticum cum Ictero), and to the Knowledge of Cell Degeneration and Cytolysis in General . . . 103

Plate VIII.

VIII. STEENSLAND, H. S. Neuroma Embryonale of the Choroid Plexus of the Cat 120

Plates IX and X.

172021

PAGE

IX. MELTZER, S. J., and SALANT, WILLIAM. Studies on the Toxicity of Bile 127

X. FLEXNER, SIMON. The Constituent of the Bile Causing Pancreatitis and the Effect of Colloids upon its Action 167

XI. LEVENE, P. A., and MANDEL, J. A. On the Carbohydrate Group in the Nucleoproteid of the Spleen 178

XII. LEVENE, P. A. On a Certain Crystalline Body Obtained on the Prolonged Digestion of Gelatine 180

XIII. OPHÜLS, W. Gastric Ulcers in Rabbits Following Resection of the Pneumogastric Nerves below the Diaphragm 181

Plate XI.

No. 2, MARCH 26, 1906.

XIV. DE WITT, LYDIA M. Morphology and Physiology of Areas of Langerhans in Some Vertebrates . . 193

Plates XII–XV.

XV. BURTON-OPITZ, R. The Effect of Intravenous Injection of Solutions of Dextrose upon the Viscosity of the Blood 240

XVI. BURTON-OPITZ, R., and MEYER, GUSTAVE M. Effects of Intravenous Injections of Radium Bromide 245

Plate XVI.

XVII. NOGUCHI, HIDEYO. The Photodynamic Action of Eosin and Erythrosin upon Snake Venom . . 252

XVIII. NOGUCHI, HIDEYO. The Effect of Eosin and Erythrosin upon the Hæmolytic Power of Saponin 268

XIX. BUNTING, C. H. Congenital Cystic Kidney and Liver with Family Tendency 271

XX. STEWART, G. N., GUTHRIE, C. C., BURNS, R. L., and PIKE, F. H. The Resuscitation of the Central Nervous System of Mammals 289

XXI. KLOTZ, OSKAR. Studies upon Calcareous Degeneration 322

No. 3, May 25, 1906.

PAGE

XXII. Madsen, Th., Noguchi, H., and Walbum, L. The Influence of Temperature upon the Rate of Reaction (Hæmolysis, Agglutination, Precipitation) 337

XXIII. Bunting, C. H. The Formation of True Bone with Cellular (Red) Marrow in a Sclerotic Aorta 365

XXIV. Knox, J. H. Mason, Jr., and Schorer, Edwin H. The Relation of Types of Diarrhœa in Children to Strains of Bacillus Dysenteriæ 377

XXV. Harvey, W. H. Studies upon the Influence of Tension in the Degeneration of Elastic Fibres of Buried Aortæ 388

Plates XVII–XIX.

XXVI. Pearce, Richard M. Experimental Myocarditis: A Study of the Histological Changes Following Intravenous Injections of Adrenalin 400

Plate XX.

XXVII. Opie, Eugene L. The Enzymes in Phagocytic Cells of Inflammatory Exudates 410

XXVIII. Ford, William W. The Toxicological Constitution of Amanita Phalloides 437

XXIX. Goldhorn, L. B. Concerning the Morphology and Reproduction of Spirochæta Pallida and a Rapid Method for Staining the Organism 451

Plates XXI and XXII.

XXX. Levene, P. A., and Beatty, W. A. On Glycylprolin Anhydride Obtained on Tryptic Digestion of Gelatine 461

XXXI. Levene, P. A., and Beatty, W. A. On the Phosphotungstates of Certain Animoacids . . . 463

No. 4, August 1, 1906.

XXXII. Thompson, R. L. A Study of Epidermal Fibrils . 467

Plate XXIII

PAGE

XXXIII. Clowes, George H. A., and Baeslack, F. W. On the Influence Exerted on the Virulence of Carcinoma in Mice by Subjecting the Tumor Materials to Incubation Previous to Inoculation . . . 481

XXXIV. Klotz, Oskar. Studies upon Calcareous Degeneration. The Relation of Experimental Arterial Disease in Animals to Arteriosclerosis in Man 504

Plate XXIV.

XXXV. Flexner, Simon, and Sweet, J. Edwin. The Pathogenesis of Experimental Colitis, and the Relation of Colitis in Animals and Man . . . 514

XXXVI. Opie, Eugene L. Solution of Tissue with Abscess 536

XXXVII. Zinsser, Hans. A Simple Method for the Plating of Anaërobic Organisms 542

Plate XXV.

XXXVIII. Flexner, Simon, and Noguchi, Hideyo. The Influence of Colloids upon the Diffusion of Hæmolysins 547

Plates XXVI–XXX.

No. 5, October 12, 1906.

XXXIX. Eyster, J. A. E. Clinical and Experimental Observations upon Cheyne-Stokes Respiration . . 565

Plates XXXI–XL.

XL. Noguchi, Hideyo. Therapeutic Experiments with Anticrotalus and Antimoccasin Sera 614

XLI. Bunting, C. H. Experimental Anæmias in the Rabbit 625

XLII. Hitchens, Arthur Parker. An Improved Syringe for Injecting Precise Amounts 647

Plate XLI.

PAGE

No. 6, DECEMBER 21, 1906.

XLIII. SIMON, C. E. A Contribution to the Study of the Opsonins 561

XLIV. WOLLSTEIN, MARTHA. The Influenza Bacillus in Inflammations of the Respiratory Tract in Infants 681

XLV. MELTZER, S. J., and AUER, John. The Effects of Intraspinal Injection of Magnesium Salts upon Tetanus 692

XLVI. JOBLING, J. W. The Occurrence of Specific Immunity Principles in the Blood of Vaccinated Calves 707

XLVII. CRILE, G., and DOLLEY, D. H. An Experimental Research into the Resuscitation of Dogs Killed by Anesthetics and Asphyxia 713

Plates XLII–XLIX.

XLVIII. NOGUCHI, HIDEYO. The Thermostabile Anti-complementary Constituents of the Blood . . . 726

THE EFFECT OF EOSIN UPON TETANUS TOXIN AND UPON TETANUS IN RATS AND GUINEA-PIGS.

By SIMON FLEXNER, M.D., and HIDEYO NOGUCHI, M.D.

(From the Rockefeller Institute for Medical Research, New York.)

The study of the action of certain photodynamic substances upon living cells, ferments, and toxins has brought out highly interesting facts. The literature upon this subject is already large and will be briefly reviewed in the paper of one of us (Noguchi) upon the action of these chemicals upon snake-venom.[1] Jodlbauer and v. Tappeiner [2] studied the action of certain fluorescent substances upon diphtheria and tetanus toxins. They found that when the toxins were mixed with 0.05 per cent. of eosin and exposed for three days to diffuse sunlight their toxicity had greatly diminished. The sample of tetanus toxin thus treated produced in 1 to 10 m. l. d. local tetanus only, while in 25 m. l. d. and over it was fatal. Huber [3] stated that the addition of one per cent. of eosin to tetanus toxin, and an exposure of six hours to the sunlight, suffice to destroy the poison, but if the mixture is kept in the dark the poison is not deprived of its toxicity. The dose used by Huber in testing toxicity was 1 m.l.d. Huber also noted a loss of hæmolytic power.

Our experiments with tetanus toxin were begun last spring and were completed before Huber's paper appeared. They embrace the study, first, of the influence of certain aniline dyes upon tetanolysin; second, the effect of eosin upon tetanospasmin; and, third, the influence of eosin upon the course of experimental tetanus in rats and guinea-pigs.

The Effects of Certain Aniline Dyes upon Tetanolysin.

Ehrlich [4] showed tetanus toxin to be composed of two poisons—the hæmolysin, tetanolysin, and the convulsive body, tetano-

[1] To appear in the next number of this Journal.

[2] Jodlbauer and v. Tappeiner, *Münch. med. Wochenschrift*, 1904, li, 737.

[3] Huber, *Arch. f. Hygiene*, 1905, liv, 53.

[4] Ehrlich, *Berliner klin. Wochenschrift*, 1898, xxxv, 273.

spasmin. Madsen,[5] proved the former to be a highly labile body. Our experiments were made with two samples of the toxin: one a dried ammonium sulphate precipitate of bouillon cultures, and the other a fresh filtrate of bouillon cultures. The first was used as a four per cent. solution; the second in full strength, sterile salt being added to produce isotonicity. The determination of initial hæmotoxicity gave the following:

TABLE I.

	Sample I.	Sample II.
0.2 c.c.	C. H	C. H.
0.15 "	"	"
0.1 "	"	"
0.07 "	"	Almost C. H.
0.05 "	Almost C. H.	"
0.04 "	Moderate H.	Moderate H.
0.03 "	Slight H.	"
0.025 "	Trace H.	Trace H.
0.02 "	"	"
0.015 "	"	"
0.01 "	No H.	No H
0.007 "	"	"
0.005 "	"	"

To two cubic centimeters of each preparation varying amounts of one per cent. eosin in saline were added; the mixtures were kept at 37° C. for one hour and tested against two per cent. washed rabbits' corpuscles. The tubes were incubated four hours at 37° C. and kept eighteen hours at room temperature.

TABLE II.

Tetanolysin 2 c.c.	Sample I.	Sample II.
Eosin 1 % 1.0 c.c.	No H.	No H.
" 0.7 "	"	"
" 0.5 "	"	"
" 0.3 "	"	"
" 0.2 "	Slight H.	Slight H.
" 0.15 "	"	"
" 0.1 "	Moderate H.	Moderate H.
" 0.07 "	Much H.	Much H.
" 0.05 "	Almost C. H.	Almost C. H.
" 0.03 "	C. H.	C. H.
" 0 "	C. H.	C. H.

[5] *Zeitschr. f. Hygiene*, 1900, xxxii, 214.

Two cubic centimeters of tetanolysin (representing about 150 m. h. d.[6]) are completely destroyed by 0.3 cubic centimeter of a one per cent. solution of eosin, in one hour in the dark, at 37° C. In other words, an eosin strength of 0.13 per cent. quickly destroyed the lysin.

Simple exposure of tetanus toxin in test-tubes to sunlight for six hours completely destroyed the lysin; and an exposure for two hours in the presence of 0.03 cubic centimeter eosin solution to two cubic centimeters of the toxin also destroyed the hæmolysin. In the dark this small quantity of eosin has no action upon the lysin. The action of the eosin in the light is very rapid, since after ten minutes' exposure the hæmolytic property may be lost.

A series of experiments were made with Grübler's methylene blue, orange G, orcein, fuchsin, and vesuvin. Berlin blue was also tried. The results may be summarized as follows: In strengths of 0.2 per cent. and above, methylene blue and vesuvin destroy tetanus hæmolysin in one hour. Quantities below 0.1 per cent. are partially destructive only; while 0.04 per cent. no longer checks hæmolysis. Fuchsin, orcein, and fluorescein are also injurious, but their limits of action were not determined. Berlin blue is without effect.

These experiments bring out the fact that aniline dyes, fluorescent ones, chiefly, have an injurious effect on the labile hæmolysin in the dark, although the effect is less than in the light.

The Effect of Eosin upon Tetanospasmin.

The fatal constituent of tetanus toxin is the convulsive agent tetanospasmin, which has an especial affinity for nervous tissues. We tested the action of eosin in the dark as well as in the light upon the convulsive principle. To one cubic centimeter of the toxin (Sample II) were added varying amounts of five per cent. eosin. The mixtures were kept one hour at 37° C. before injection in the right gluteal region into white rats, weighing about 90 grams each. The animals were kept in a room diffusely lighted; the cage was partly shaded. The toxin employed was fatal in four days in a dose of 0.0025 cubic centi-

[6] Minimal hæmolytic dose.

meter, the first symptoms of tetanus appearing in twenty-four hours; 0.0005 cubic centimeter was the minimal tetanic dose, and 0.001 caused strong tetanus but was not fatal. Doses of about 0.4 cubic centimeter caused death in from 24 to 48 hours. Hence one cubic centimeter of the toxin contained about 400 m. l. d. and 2000 m. t. d.[1] for the rats.

Experiments. Rat 1. Toxin 1 c.c. plus 5 % eosin 1 c.c. 1 hour at 37° C in thermostat. Injection of full amount, 10 A.M., Oct. 13. Next day, no tetanus, nor did tetanus develop later. The leg was swollen for several days as result of injection.

Rat 2. Toxin 1 c.c., 5 % eosin 0.5 c.c. Same conditions. Entire quantity injected, Oct. 17. No effect except swelling at injection point.

Rat 3. Toxin 1 c.c., 5 % eosin 0.3 c.c. Same conditions. Injection, Oct. 19, 10 A.M. Oct. 20, slight tetanus; increased next day. Oct. 30, still marked tetanus of injected leg. No general tetanus. Nov. 3, found dead. Lived 15 days.

Rat 4. Toxin 1 c.c., 5 % eosin 0.1 c.c. Same conditions. Oct. 13, 10 A.M., injection. Oct. 14, slight tetanus; next day, marked tetanus. Oct. 19, tetanus increased; dead, Oct. 21. Lived 9 days.

Rat 5. Control. Toxin 1 c.c. Oct. 13, 10 A.M. Next day, marked tetanus. 5 P.M., dead. Lived 29 hours.

These protocols show that eosin in solutions exceeding one per cent. in strength quickly destroys tetanospasmin in the dark, and in 0.6 per cent. solutions reduces greatly the activity of the poison. The chronic nature of the tetanus which the modified poison produces is a striking and interesting occurrence. To what this change is to be attributed—whether to influence of the eosin upon the animal organism or to peculiar changes in the poison itself—has not been determined with accuracy. We shall return to this topic.

The Effect in the Animal Body of Eosin upon Tetanus Toxin.

In this series of tests tetanus toxin and eosin were injected simultaneously but separately into the rat.

Experiments. Rat 1. Oct. 20, 10 A.M. Toxin, 1 c.c. 1 c.c. 5 % eosin immediately injected about the site of introduction of the poison. Oct. 21, slight tetanus, which increased until death on Oct. 24. Lived 4 days.

Rat 2. Same date. Toxin 0.1 c.c. 1 c.c. 5 % eosin immediately injected about toxin site. Oct. 22, no tetanus. Oct. 23, very slight tetanus. Oct. 24, marked tetanus. Increase until death on 7th day.

[1] Minimal tetanic dose

Rat 3. Oct. 27, 11 A.M. Injected toxin, 0.05 c.c., and immediately 1 c.c. 5 % eosin. No tetanus, Oct. 30. On 31st, suspicion of tetanus. Next day, tetanus increased, and then progressive increase until death on 8th day.

Rat 4. Oct. 28, 10 A.M. Toxin, 0.01 c.c. and eosin as before. Oct. 30, suspicion of tetanus; 31st, increased slightly; in next days, further increase. Nov. 10, dead. Lived 13 days.

Rat 5. Oct. 29. Toxin, 0.01 c.c., injected into right leg and 5 % eosin solution 1 c.c. injected into left leg. Oct. 30, right leg not used; Oct. 31, marked tetanus. Nov. 3, dead. Lived 5 days.

In this series of experiments the effect of eosin upon the course of the tetanus consisted in delaying the onset of symptoms and in prolonging the period of intoxication. Since the toxin employed caused, in control animals, in doses of 0.0025 cubic centimeter, tetanic symptoms to appear in twenty-four hours, the delay of symptoms in the eosin-treated animals is at once apparent; while the prolongation of life is also well brought out by the protocols. However, none of the animals of this series was actually saved. And in view of this fact the question arises whether, after all, eosin exerts a destructive influence upon the toxin *in vivo* at all comparable with its effect *in vitro*. Since, in all but one rat, the eosin injection was made about that of the toxin, and since eosin alone in five per cent. solution causes œdema of the tissues, perhaps changes in absorption may account for the delay of onset of the symptoms and fatal effect. But this explanation does not entirely account for the effect observed, as in Rat 5 the injection of the poison in one side and the eosin in the other side of the body was made and yet the fatal result was delayed.

The Influence of Eosin upon the Course of Tetanus in Rats and Guinea-Pigs.

It is recognized that poisoning with tetanus toxin is not an exact equivalent of infection with tetanus bacilli, for while in the first the full fatal dose of poison is at once available for fixation by the tissues, in the second the appearance of the first symptoms of tetanus may not exactly coincide with the presence of a fatal quantity of the poison in the body. If, therefore, eosin is capable of modifying tetanus poison in such a manner as to

diminish its toxicity when the two are kept in contact for a short time at body temperature, even in diffuse light and in the dark, the important question arises whether this action may be exerted in the body to an extent sufficient to rescue infected animals. To imitate natural conditions more closely silk threads carrying tetanus spores but no toxin were introduced beneath the skin of the right thigh. Some of the animals remained untreated; others were treated with eosin in various ways.

Experiments. Rat 1. Control. Inoculated Nov. 1, 10 A.M. Nov. 4, first appearance of tetanus. Nov. 5, marked tetanus. Dead, Nov. 7. Lived 3 days after the appearance of tetanus.

Rat 2. Control. Nov. 5, inoculation as above. Nov. 8, tetanic. Death, Nov. 11. Survived first appearance of symptoms 3 days.

Rat 3. Nov. 1, 10 A.M. Thread introduced as before. Immediately afterwards 1 c.c. 5 % eosin injected in the tissues above the thread. Nov. 2, œdema; leg not used. Nov. 5, no tetanus; small necrosis at site of injection. Dec. 1, no tetanus; animal normal.

Rat 4. Nov. 1, thread introduced. Nov. 3, first appearance of tetanus. 1 c.c. 5 % eosin injected in same leg. Nov. 4–6, tetanus increased. Nov. 11, dead. Lived 9 days after first appearance of symptoms.

Rat 5. Nov. 1, thread introduced in right leg. Nov. 4, slight tetanus. 1 c.c. 5 % eosin injected in left leg. Tetanus increased. Death on Nov. 8. Survived first symptoms 4 days.

Rat 6. Nov. 1, thread introduced. Nov. 2, no tetanus; 0.5 c.c. 5 % eosin injected about the thread. Nov. 3, no tetanus; eosin injection. Nov. 4, no tetanus; injection repeated. Same on Nov. 5, 6, 7, 8. No further injections. Dec. 3, no tetanus. Animal normal.

Rat 7. Nov. 1, thread introduced. 0.2 c.c. 5 % eosin injected every 24 hours as in Rat 6. Nov. 5, slight tetanus, which gradually progressed until Nov. 12, death. Survived first appearance of symptoms 7 days.

Rat 8. Nov. 1, thread introduced. 0.5 c.c. 5 % eosin injected intraperitoneally every 24 hours for four days. Nov. 5, tetanus appeared; Nov. 12, dead. Survived first symptoms 7 days.

This series of experiments indicates that the power of eosin to destroy or modify the tetanic poison in the body, while marked is not absolute. However the eosin is brought into the body of the infected animals, it causes an increase in the period of intoxication and delay in the supervention of death. If the eosin solution is sufficiently concentrated and is brought into relation with the focus of development of the tetanus spores, it is capable not only of modifying the tetanus but of wholly sup-

pressing its development. The manner of this action is not yet wholly worked out, and hence we cannot at present say whether the suppression affects the germination of the spores or merely the detoxication of the poison as it is produced. This part of the subject, as well as still other phases of it, as, for example, the value of combined eosin and anti-toxin treatment of animals poisoned with tetanus toxin and infected with tetanus spores, the effects of still other photodynamic substances, and the influences of the aniline injections upon local absorption, are now being studied. The influence of eosin upon the production of tetanus toxin *in vitro* is also being determined.

A parallel series of experiments to the latter, carried out on the more highly sensitive guinea-pig, will now be given briefly. The pigs weighed 300 grams each.

Experiments. Guinea-pig 1. Control. Nov. 21, thread introduced. Nov. 22, slight tetanus. Nov. 23, marked tetanus; dead in night. Lived 60 hours.

Guinea-pig 2. Control. Nov. 24, 10 A.M. Tetanus spores. Nov. 25, marked tetanus. Nov. 25, 9 P.M., dead. Survived 36 hours. A third control lived 2½ days.

Guinea-pig 3. Nov. 21, 10 A.M. Spores introduced. 1 c.c. 5 % eosin immediately injected into same leg. Nov. 22, leg swollen; no tetanus. Nov. 23 to 28, no tetanus. Animal emaciating; dead of marasmus, Dec. 1. No symptom of tetanus at any time.

Guinea-pig 4. Nov. 21, 10 A.M. Spores; at same time, 1 c.c. 5 % eosin in opposite leg. Nov. 22, marked tetanus. Died during the night of 23d. Survived 60 hours.

Guinea-pig 5. Nov. 21, 10 A.M. Spores. Nov. 22, slight tetanus. 1 c.c. 5 % eosin injected in locality of thread. Nov. 23, no progress of tetanus. Nov. 24, tetanus increased; no further change until Nov. 27. Animal weaker and tetanus increased. Nov. 28, dead. Survived 6 to 7 days.

Guinea-pig 6. Nov. 21, 11 A.M. Same experiment as No. 5, but the eosin injected into opposite leg. Survived 3 days.

Guinea-pig 7. Nov. 21, 10 A.M. Spores. Nov. 22, slight tetanus. 1 c.c. 5 % eosin injected into same leg. Nov. 23, tetanus increased; eosin injection, Nov. 24, eosin injection. Nov. 25, symptoms progressing, eosin injection. Nov. 26, *idem*. Nov. 27, dead. Survived first symptoms 5 days.

This series of experiments shows that even in the highly sensitive guinea-pig tetanic symptoms may be restrained, provided the eosin is brought into close relationship with the developing tetanus poison before it is fixed by the nervous tissues or reaches the general circulation.

ON THE PHYSIOLOGY OF HEART-BLOCK IN MAMMALS, WITH ESPECIAL REFERENCE TO THE CAUSATION OF STOKES-ADAMS DISEASE.

By JOSEPH ERLANGER.

(*From the Physiological Laboratory of the Johns Hopkins University.*)

PLATES I.–IV.

PART II.

ON THE PHYSIOLOGY OF HEART-BLOCK IN THE DOG.

INTRODUCTION.

At the present time two views are held with regard to the path taken by the impulse which normally causes the various chambers of the heart to beat. According to the older view, that of the supporters of the neurogenic theory, the impulse arises in the automatic ganglia of the heart and is distributed to the muscle cells through the medium of nerves. According to the more recent view, which is supported by the upholders of the myogenic theory, the impulse arises in the automatically rhythmical musculature of the great veins and is propagated to the various chambers of the heart through their musculature after the nature of a peristalsis.

The literature which has appeared in the support of one or the other of these theories has reached such stupendous proportions that it would be impossible to review it here. An account of it will be found in the recently written reviews of Gaskell,[1] Hofmann,[2] and Langendorff.[3] Here we wish to refer to but one phase of the subject, that which is concerned with the conduction of the impulse from the auricles to the ventricles of the mammalian heart.

[1] Gaskell, *Schäfer's Text-book of Physiology*, 1900, ii, 169.
[2] Hofmann, *Schmidt's Jahrbücher*, 1904, cclxxxi, 113.
[3] Langendorff, *Ergebnisse der Physiologie*, 1902, i, pt. ii, 263.

Undoubtedly the greatest stumbling-block for the supporters of the myogenic theory consisted in the view, which was maintained by the anatomists, that in mammals the musculatures of the auricles and ventricles are completely separated from one another by connective tissue. Until it could be shown that this is not the case the myogenic theory could have no sound basis, at least in so far as it concerned the mammals. To His, Jr., belongs the credit of being the first to find a muscular connection,[4] the existence of which means so much to the supporters of the myogenic theory. His has shown, and this has since been abundantly confirmed,[5] that in mammals there is a narrow band of muscular tissue which joins the septum of the auricles with the septum of the ventricles. The anatomy of this bundle, which is rightly termed the auriculo-ventricular bundle of His, has been studied most carefully by Retzer. A free translation of his description of it in man follows. ". . . When viewed from the left side the bundle lies just above the muscular septum of the ventricles and below the membranous septum. In some hearts the muscular septum is so well developed that it envelopes the bundle; it is then difficult to find it. But occasionally it can be seen directly by means of transmitted light. From the left side the bundle can be followed posteriorly no farther than the right fibrous trigone, for here the connective tissue becomes so dense that it is difficult to dissect it away. The impression is therefore received that this mass of connective tissue forms the point of insertion of the bundle. The bundle may be followed anteriorly until it becomes intimately mixed with the musculature of the ventricles."

"When viewed from the right side of the heart the bundle cannot be seen because it is covered by the mesial leaflet of the tricuspid valve whose line of attachment passes obliquely over the membranous septum. To expose the bundle, this leaflet must be cut away from the membranous septum. Then, if the endocardium is removed from the posterior part of the septum of the auricle

[4] His, Jr., *Arbeiten aus der med. Klinik zu Leipzig*, 1903; Ref., Breaunig.

[5] Retzer, *Arch. für Anat.*, 1904, p. 1; Breaunig, *Arch. für Phys.*, 1904, p. 1; Humblet, *Arch. internat. de phys.*, 1904, i, 278.

up to the membranous septum, the posterior part of the auriculo-ventricular bundle will be exposed. If, in addition, the membranous septum be removed, the bundle may be traced from the point to which it could be followed when viewed from the left side as it passes posteriorly over the muscular septum. In the region of the auriculo-ventricular junction it loses its compactness, the fibers divide, and the bundle seems to fork. One branch passes into the superficial part of the valve musculature which descends from the auricles, and the other branch passes directly into the musculature of the auricle."

"Briefly, the auriculo-ventricular bundle runs posteriorly in the septum of the ventricles about 10 mm. below the posterior leaflet of the aortic semilunar valves; with a gentle curve it passes posteriorly just over the upper edge of the muscular septum and sends its fibers into the musculature of the right auricle and of the auricular valves. . . . In the heart of the adult the bundle is 18 mm. long, 2.5 mm. wide, and 1.5 mm. thick."

The anatomy of the auriculo-ventricular bundle in the other mammals is practically the same as in man, but in them its course can be determined only by microscopical examination of sections.

As the auriculo-ventricular bundle of His represents the only demonstrable muscular connection between auricles and ventricles in the heart of mammals, it follows that if the myogenic theory is correct, destruction of this bundle should prevent the passage of the impulse from auricles to ventricles. This is the *sine qua non* of the myogenic theory.

The first attempt to destroy this connection between the auricles and the ventricles in the mammalian heart was made by His, Jr.[6] In this he seems to have succeeded. He states that section of the auriculo-ventricular bundle in the rabbit is followed by complete independence of the rhythms of the auricles and the ventricles. But as he has never made more than a pre-

[6] His, Jr., "Bericht des dritten internationalen Physiologenkongress in Bern," *Zent. für Phys.*, 1895, ix, 469; and *Deutsches Arch. für klin. Med.*, 1899, lxiv, 316.

liminary report of these experiments, and as his tracings have never been published, practically no notice has been taken of his work.

Very recently, indeed after our experiments had been begun, we came into the possession of an article by Humblet,[7] in which are described some experiments performed with the object of testing the effect of section of the auriculo-ventricular bundle in the heart of the dog. Humblet did not succeed in accomplishing this with the heart *in situ*. He gives but one tracing obtained after section of His's bundle in an excised heart. This tracing shows that complete heart-block resulted.

After a preliminary notice of the experiments herein recorded had been sent abroad for publication there appeared in *Pflüger's Archiv* an article by Hering in which are described the results of cutting the auriculo-ventricular bundle in the perfused heart.[8] Like His and Humblet, Hering found that section of the bundle produced complete heart-block. But Hering carried the research one step further, since he shows that section of the bundle does not interfere with the action of the accelerator nerve on the ventricles.

These are the only researches which bear directly upon this question. Therefore, when our interest in the problem of heart-block was aroused by the studies reported in the first part of this paper, we were impressed with the need of a series of experiments which would put the question of the mode of conduction from auricles to ventricles in the mammalian heart to a thorough test, in the hope that the solution of this problem might throw some light on the pathogenesis of heart-block in man. It was with this object that the experiments to be reported were performed.

METHODS.

In the first experiments an attempt was made to pass a ligature, carried by a fine curved needle, about the region through which the auriculo-ventricular bundle passes. Of the seven experiments performed, in only one was heart-block obtained.

[7] Humblet, loc. cit.

[8] Hering, *Pflüger's Archiv*, 1905, lvii, 97.

Examination of the protocols (Exp. 1–7) will show that this was the only experiment (Exp. 3) in which the ligature had been so placed as to include the region of His's bundle.

An attempt was next made to devise a clamp in which the region of the auriculo-ventricular bundle could be compressed just as the auriculo-ventricular junction of the cold-blooded heart may be compressed in Gaskell's clamp.[9] In the first form of clamp devised, the principle of the carpenter's vise was applied. Two bars were each armed at one end with projecting points which were curved in appropriate directions. The points were inserted into the heart so that presumably one lay on each side of the region which contains His's bundle. The bars were so arranged that they could then be conveniently joined together by means of two parallel screws. By driving nuts on these screws the bars could be made to approach one another in parallel, thus compressing the tissue included between them.

Seven experiments were performed with this clamp. In the protocols of these (Exp. 8–14) it may be seen that in two cases (Exp. 8 and 14) heart-block was obtained. These were the only instances in which the clamp had been so placed as to include in its grasp the bundle of His. These results were not considered satisfactory because they could not be nicely controlled and because of the difficulty experienced in placing the clamp properly.

Finally, the following thoroughly satisfactory clamp was devised (Fig. 19.). A fish-hook provided with a very fine barb is bent into the shape of the letter "L." The blunt end of the hook is soldered into the end of a brass bar. That half of the bar to which the hook is attached is square on cross-section and serves as a guide for the brass block, A. The other half of the bar has a fine thread cut in it and carries a nut, B, which may be put in movable connection with the brass block. The edge of the brass block which is directed toward the short arm of the "L" is carefully rounded and is just long enough to fit accurately between the long arm of the "L" and the barb at the end of the hook. The size of the clamp reproduced in Fig. 19 is convenient for dogs.

[9] Gaskell, *Philosophical Transactions of the Royal Society*, 1882, pt. ii, 994.

To put the clamp on the heart, the heart is first freely exposed by removing the upper two-thirds of the sternum with the adjacent portions of the ribs. The pericardium is opened and stitched to the chest wall so as to lift the heart well toward the surface of the wound. Then, by blunt dissection, the epicardium is split along the line where it passes from the fatty tissue in the auriculo-ventricular groove to the right side of the aorta, a few millimeters posterior to a line which is parallel to the axis of the animal and which passes through the origin of the right coronary artery. Approximately, it is the line which forms the right edge of the mass of fat which covers the anterior aspect of the root of the aorta. By lifting the loose connective tissue away from the wall of the aorta along this line it is possible to arrive at a place immediately above the membranous septum of the ventricles. The point of the hook is set in this place and, while directed obliquely downward and toward the lumen of the aorta, it is thrust into the left ventricle. If the point has been set in the proper place it will be found that it has entered the heart near the line of junction of the right with the posterior leaflet of the aortic valve and usually a few millimeters below the uppermost point of attachment of this valve. The bar is now turned so that the point of the hook is directed caudally, with the short arm of the "L" parallel and close to the ventricular septum. The length of the short arm of the hook has been so chosen that the point will now lie just over the place at which it should be made to enter the ventricular septum. This place is on a perpendicular erected at the middle point of the posterior edge of the membranous septum and a few millimeters removed from this edge. By moving the bar until it lies parallel to the first part of the arch of the aorta, and by pressing the point of the hook in the direction of the ventricular septum, the short arm

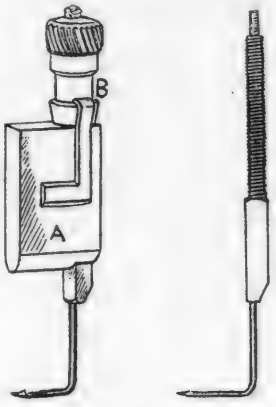


FIG. 19.

of the "L" will perforate the septum at about the right place. The point will enter the right ventricle from 3 to 6 mm. below the auriculo-ventricular junction and somewhat posterior to the anterior edge of the mesial leaflet of the tricuspid valve. The brass block and the nut are then attached to the bar. It might be added that the main trunk of the right coronary artery should not be included in the grasp of the clamp.

This procedure is not difficult: it is only necessary to become familiar with the position which the clamp should occupy, in order to be able to apply it successfully. Since the clamp just described was perfected, heart-block has been produced with it in every one of many experiments.

In the first experiments the contractions of the auricles and ventricles were recorded with the myocardiograph of Roy and Adami. But this instrument occupied so much of the available room that when it was in place great difficulty was experienced in manipulating the clamp. We finally found it most convenient to record the auricular contractions by means of a tambour system. A thread attached to the auricular appendix was made to pull against the head of a receiving tambour. The auricular contractions were therefore recorded as down strokes. A manometer connected with the central end of the carotid artery served to record at one time the movements of the left ventricle through the pulse-waves caused by its contractions, and the arterial pressure. This method has the disadvantage of not showing the exact sequence of auricular and ventricular contractions. The relation which the beginning of the pulse-waves recorded by the manometer bears to the beginning of the ventricular systoles will vary to some extent with the rate of transmission of the pulse-waves and with the time intervening between the beginning of ventricular contraction and the opening of the semilunar valves. As, in this paper, no stress will be laid on small differences in time, these errors will certainly be negligible. For the most part the mercury manometer was used. In a few experiments the Hürthle manometer was used. The time, in fifths of seconds, was recorded on the base line.

All of the animals were anæsthetized with morphine and ether.

RESULTS.

It is natural that in a research of this kind the same conditions cannot be reproduced with certainty in each experiment. In different experiments the hearts might not have been in the same condition. The clamp might not always have been applied in the same way; it might, for instance, have done permanent injury to the conducting tissue at the moment it was inserted, or it might have been placed in such a position that the conducting tissue was more or less protected from compression. In one experiment the pressure might have been applied more rapidly than in another. Often the same results were not obtained while releasing the pressure on the conducting tissue as had been obtained while compressing the tissue. As a rule, during the return to normal after clamping, more intermediate stages have been obtained than while clamping. This result is to be explained by the fact that the recovery from the injury produced by the clamping is slower than the production of that injury. Naturally, variable results corresponding with these variable and other unaccountable conditions have been obtained in the different experiments. Therefore, in order to present a complete picture of the effect of clamping the region of the auriculo-ventricular bundle, it will be necessary to bring together comparable results of different experiments. Most of these results were obtained while clamping, but a few of them were obtained only while unclamping.

THE EFFECT ON THE SEQUENCE AND RHYTHM OF THE HEART-BEAT OF GRADUALLY COMPRESSING THE REGION OF THE AURICULO-VENTRICULAR BUNDLE.

If the clamp has been properly adjusted on the heart, the heart will continue to beat with undisturbed sequence and rhythm. Its rate may be somewhat increased. Upon cautiously tightening the clamp, the first obvious effect may be an increase in the period intervening between the beginning of an auricular contraction and the beginning of the ventricular contraction, *the intersystolic period*, of the same cardiac cycle. The duration of

the intersystolic period may then gradually increase in the successive cycles until eventually the ventricles fail to respond to one of the auricular contractions (Fig. 20, G). Immediately following this ventricular "silence," the intersystolic period again becomes short and it again increases, at first slowly, then rapidly just before the next ventricular silence. At first the ventricular beats may be dropped rather irregularly: the longest interval between two ventricular silences has been twenty-seven beats (Fig. 20, G). But soon the ventricular silences recur regularly: the same small number of normal beats may always be interposed between them. Just before each ventricular silence the intersystolic period lengthens quite rapidly. Stages have been obtained which show one ventricular silence recurring regularly in ten, nine, eight, seven, six, five, four, three, and two auricular beats. (Fig. 20; F, E, D, C, B, A.)

Many of the results above referred to were obtained in an experiment in which the Hürthle manometer was used to record the arterial pressure on a rapidly moving surface (Exp. 27). The results obtained in this experiment indicate that the conducting tissue had not been nicely included in the clamp, or that it had been protected by some other structure. This is borne out by the fact that to obtain the different stages of block it was found necessary in this experiment to tighten the clamp through a considerable distance, and that the last stage usually obtained while clamping was attained with great difficulty. These results are in sharp contrast with those of Exp. 31.

But usually upon clamping the normal sequence will more or less abruptly give way to a partial block in which the ventricles respond only to every other beat of the auricles, giving a 2:1 rhythm (Fig. 21). Frequently before the final change occurs there may be some irregular alternation between the normal sequence and the 2:1 rhythm. With further clamping a 3:1 rhythm may be established, preceded, usually, by some preliminary alternation between a 2:1 and a 3:1 rhythm. This will then give way to complete block: the beats of the ventricles will be totally independent of those of the auricles and very much slower than them. The inverse order of changes in rhythm may be obtained when the clamp is unscrewed, provided care has been taken to prevent permanent injury of the tissue included in the

clamp. These changes in the conductivity at the auriculo-ventricular junction of the dog's heart may be obtained as regularly and as promptly as in experiments in which the auriculo-ventricular junction of the cold-blooded heart is compressed in Gaskell's clamp. The stages between normal sequence and complete block cannot, as a rule, be maintained over a long period of time. In the course of time, even though the amount of pressure exerted by the clamp remain the same, a partial block will pass into complete block or into the normal state, according as the clamp had previously been tightened or loosened. But it is possible to keep the block complete for over an hour and still have the normal sequence return promptly upon loosening the clamp (Exp. 15).

The picture presented by the exposed heart with complete block at the auriculo-ventricular junction may readily be formed. But from the standpoint of the diagnosis of the condition in the human being it is important to know exactly what may be seen. The ventricles, of course, beat much more infrequently than the auricles. After the ventricles have emptied themselves, it may be seen that each contraction of the auricles sends into the former a distinct wave, upon the subsidence of which the volume of the ventricles is seen to have been considerably increased. In this way the ventricles are rhythmically distended by the contractions of the auricles. Likewise, it may be seen that each contraction of the auricles sends a wave into the great veins. After two or three such waves have occurred the ventricles contract, but this contraction bears no constant time relation to the contractions of the auricles. These two perfect and independent rhythms which are characteristic of complete block may therefore be seen upon inspection of the great veins or of the ventricles. A cardiogram obtained by resting the receiving tambour directly upon the ventricles of the heart while in complete block is reproduced in Fig. 22. In it the beats of the auricles and of the ventricles may be distinctly seen. This tracing should be compared with Figs. 1 and 2 (Part I), in which are reproduced comparable tracings obtained from man.

The rate of the ventricles when beating independently of the auricles has varied within rather wide limits. As is shown in Table XVI, at the beginning of an experiment it has varied between 34.8 and 69.8, the average being 54.1. Toward the close of the experiments it has been somewhat slower. No attempt has been made to determine the cause of this variability, but the temperature of the exposed heart is probably one of the most important factors. As may be seen in the table, the inherent rate of the ventricles has always been considerably less than that of the auricles, the average ratio, $\frac{A}{V}$, being 3.05. (Compare with values obtaining in man.)

TABLE XVI.

SHOWING THE AURICULAR AND VENTRICULAR RATES OF THE DOG'S HEART DURING COMPLETE BLOCK AT THE BEGINNING OF EXPERIMENTS, INCLUDING, FOR COMPARISON, A FEW ESTIMATIONS IN MAN.

No. of Exp. or Case.	No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	$\frac{A}{V}$	Max. Pres.	Min. Pres.	Remarks.
14	8	69.0	34.8	32.0	74.0	130.0	3.7	41	21.5	
15	20	86.0	69.8	62.0	86.0	216.0	3.1	59	49	
18	8	43.0	55.8	25.3	43.0	177.0	3.2	79	58	
19	16	70.6	68.0	37.7	70.6	160.2	2.4	76	70	
20	8	50.0	47.8	26.4	50.2	157.7	3.3	94	88	
21	8	53.5	44.9	21.0	53.5	117.8	2.6	116	98	
24	7	36.3	57.8	21.3	36.3	175.8	3.04	—	—	
Average.			54.1			162.1	3.05			
Case I.			22.4			70.6	3.55			Extremes under normal conditions
" "			31.0			84.6	2.73			
" II.			31.7			89.3	2.82			Only determination.

THE EFFECT ON THE BLOOD-PRESSURE OF VARYING THE SEQUENCE OF THE HEART-BEAT.

With each change in rhythm the blood-pressure is materially affected. (See protocols in the Appendix.) It falls whenever the rate of the ventricles is decreased and rises whenever the rate of the ventricles is increased. The results obtained in the different experiments vary considerably among themselves. It

will, however, be seen that a fair pressure may be maintained even in complete block. (See Fig. 21.)

Unfortunately, no systematic study was made of the variations of the systolic and diastolic pressures which are associated with the various changes in the auriculo-ventricular rhythm. However, a clue to the nature of these pressure changes may be obtained from examination of the blood-pressure record made with the Hürthle manometer (Fig. 20). It may be seen that in the occasional ventricular silences the systolic pressure is not materially affected, whereas the diastolic pressure falls decidedly. (Compare with page 722 of Part I.)

THE INFLUENCE OF THE EXTRINSIC CARDIAC NERVES ON THE AURICLES AND VENTRICLES IN VARIOUS STAGES OF HEART-BLOCK.

Vagus Effects.—In any stage of partial block the auricles and the ventricles may be completely inhibited by stimulation of the vagus nerve: inhibition is obtained at least as easily as under normal conditions. (Fig. 23, A and B). But when the block is complete, stimulation of the peripheral end of the vagus (Figs. 23, C, and 24), although it inhibits the auricles, usually has little effect upon the rate of the ventricles. Indeed, in one experiment (Exp. 8) the rate of the ventricles increased during vagus stimulation. Occasionally a slight diminution in the ventricular rate results. Furthermore, when the heart-block is complete, stimulation of the vagus nerve has but little or no influence upon the force of the ventricular beats, as judged of by variations in the arterial pressure. Thus, during stimulation of the vagi, the arterial pressure may not be affected at all or it may fall slightly.

How much of this decrease of the arterial pressure is to be attributed to the falling away of the filling effect of the auricular contractions, and how much to a vagus effect upon the force of the ventricular contractions, cannot be determined. But it is certain that the former factor is not unimportant. Thus it may be seen in Fig. 25 that the ventricular contraction which follows immediately upon an escape beat of the auricles produces

a very much larger pulse-pressure than the other ventricular contractions.

The loss of the influence of the vagi over the ventricles is always exactly synchronous with the establishment of complete heart-block. This association is so constant that the reaction of the heart to stimulation of the vagus nerve may be used as a test to determine the presence or absence of complete block.[10]

In two experiments (Exp. 19 and 30) a 2 : 1 rhythm has temporarily changed into a 1 : 1 rhythm during recovery from vagus inhibition. This change was in all probability associated with a slowing of the auricular rate; although it may possibly have resulted from an increase in the conductivity of the heart muscle which, according to Gaskell,[11] may follow vagus stimulation in the tortoise. Stimulation of the vagus nerve during partial heart-block has never increased the resistance to the passage of impulses between auricles and ventricles—at least it has never caused a change in the direction of complete block.

When the block is complete, section of both vagi has no, or but a very slight, effect upon the rate of the ventricles, although the usual change in the rate of the auricles may occur (Exp. 15). In one experiment (Exp. 19), after having cut one vagus, freezing and thawing of the intact vagus nerve had no effect upon the rate of the ventricles. It should, however, be stated that in all of these experiments, excepting Exp. 15, the tone of the vagus center was nil, if we may use as an index of the degree of tone the effect of section of both vagi on the rate of the auricles. In the single exception, section of both vagi increased the rate of the auricles from 213 to 245 beats per minute. The ventricular rate increased but 2.1 beats per minute—from 46 to 48.1.

With but a single exception, section of both vagi has had no effect upon the force of the ventricular contractions as judged of by the effect on the blood-pressure. In the single exception,

[10] Krehl and Romberg (*Schmiedeberg's Archiv*, 1892, **xxx**, 49) have noted the same association in cases where the block was produced by crushing all of the tissue in the auriculo-ventricular junction. We have since met with exceptions to this rule in 3 : 1 rhythms (Erlanger and Hirschfelder, *Amer. Jour. of Physiol.*, Jan., 1906.)

[11] Gaskell, *Jour. of Phys.*, 1883, iv, 96.

which has been mentioned above, section of both vagi was followed by a marked and prolonged rise of the blood-pressure. We have not been able to duplicate this result.

Accelerator Effects.—When the heart-block is complete, stimulation of the accelerator nerve, as a rule, increases the rate of the ventricles as well as that of the auricles[12] (Exp. 20 and 21). The increase in the rate of the ventricles may be as great proportionally as that of the auricles. Indeed, in some tests (see Exp. 20 and 21) stimulation of the accelerator nerve has caused a decided increase in the rate of the ventricles and no change, or even a slight decrease, in the rate of the auricles. These last results were usually obtained when strong stimuli were employed, and it seems probable that the anomaly was caused by an escape of the stimulus, resulting in either direct or reflex inhibition of the auricles. This association therefore serves as a beautiful illustration of the fact that in complete heart-block the ventricles are but little affected by impulses arriving in the heart through the vagi. But the fact that the auricles are affected by the escape of stimulus, whereas the ventricles are not so affected, prevents the formulation of a positive conclusion as to the relative effect on the auricles and ventricles of stimulation of the accelerator nerve.

THE INFLUENCE OF OTHER CONDITIONS ON THE RATE OF THE VENTRICLES IN COMPLETE HEART-BLOCK.

The rate of the ventricles when beating independently of the auricles is not materially affected by variations of the blood-pressure produced by compression of the abdominal or thoracic aorta (Exp. 14 and 15), nor by extreme hæmorrhage (Exp. 21).

In one experiment (Exp. 15) the effect was tested of cutting both splanchnics. But this test was made near the close of a prolonged experiment, when the blood-pressure was low, and the heart-rate very slow—probably a temperature effect. The results of this test are, therefore, of but little value.

[12] Hering (loc. cit.) has recently noted that stimulation of the accelerator nerve accelerates the rate of the ventricles when they are beating independently of the auricles.

General asphyxia carried to the convulsive stage does not materially affect the rate of the ventricles in complete block (Exp. 15 and 18). If there is any change, it is in the direction of an increase in rate.

The blocking of one coronary artery, either the right or the left, by Porter's method [13] (Exp. 19 and 20) has not obviously altered the rate of the independent ventricles. In one experiment (Exp. 20) no marked change in the rate of the ventricles occurred after the left coronary had been plugged for four minutes. The ventricles will continue to beat for many minutes after excision of the heart. It is possible that these results are to be accounted for by a low temperature of the heart from prolonged exposure, rather than by an increase in the resistance of the ventricles when they are no longer controlled in the normal way.

UNUSUAL SLOWING OF THE VENTRICULAR RATE UPON CLAMPING.

In many experiments (Exp. 21, 22, 23, 26, 30, and 31) while carefully tightening the clamp the ventricular rate alone has abruptly become remarkably slow (see Fig. 26). As a rule, the slowing of the ventricular rate is most marked at the very onset of this phenomenon. The interval between two ventricular contractions has upon several occasions been as long as twenty seconds. The ventricular rate then gradually increases until a rate obtains which is usual for heart-block. The auricular rate does not seem to be affected. The blood-pressure may fall almost or quite to zero.

This condition is a most remarkable one to witness. Usually while carefully tightening the clamp the ventricles stop beating without warning. The auricles continue to beat with an apparently unaltered rate. With each contraction of the auricles there is a sudden, slight increase in the volume of the ventricles. The ventricles thus enlarge with each contraction of the auricles until their distension becomes really huge. The veins, too, are distended and pulsate. Respiratory convulsions may begin.

[13] Porter, *Jour. of Exper. Med.*, 1896, i, 60.

Witnesses are almost convinced that the experiment has come to a close when, it may be after more than twenty seconds, the ventricles suddenly empty themselves with one great effort. Then there succeeds another long interval during which the ventricles remain perfectly flaccid and are gradually distended by the contractions of the auricles. But usually this pause is not quite so long as the first one, and the succeeding periods become shorter and shorter until the state usually seen in heart-block is established. Although the condition appears to be extremely grave, death has never resulted from it.

In almost every instance in which marked slowing of the ventricular rate occurred it was not preceded by any of the stages of heart-block, although the usual care in tightening the clamp was, as a rule, observed. Occasionally it was preceded by a few 2 : 1 or 3 : 1 cycles. On the other hand, instances were observed in which heart-block developed just as abruptly, but in which the change was not preceded by this remarkable slowing of the ventricles (Exp. 28). In a few experiments (Exp. 31) the maximum slowing of the ventricles did not come on at once. In these the maximum slowing was reached through one or two ventricular cycles of increasing duration. Very rarely in instances in which the block was slowly established, the ventricular rate was somewhat slower at the moment of onset of complete block than a little later.

In every instance in which this phenomenon of marked preliminary ventricular slowing was observed, complete heart-block was found to exist when the ventricular rate became constant. Upon releasing the clamp after the conditions have become constant, the recovery to normal usually occurs through short stages of 3 : 1 and 2 : 1 rhythms, provided the conducting structures have not been severely injured.

At post-mortem examinations of these cases in which marked slowing of the ventricular rate has occurred, the impression is usually received that the auriculo-ventricular bundle has been unusually favorably exposed to the action of the clamp. We are, however, not at all certain as to the cause of this phenomenon, and therefore a discussion of the matter will be deferred until a

special investigation of it has been made. However, the protocols of a few experiments that have thus far been performed in the effort to throw light upon this subject will be found in the Appendix[14] (Exp. 22, 23, 24, 25, 26, 27, 30, and 31).

THE POSITION OCCUPIED BY THE CLAMP IN SUCCESSFUL AND UNSUCCESSFUL EXPERIMENTS.

In every experiment in which heart-block has been obtained it has been found at autopsy that the clamp had undoubtedly embraced the region in which the auriculo-ventricular bundle lies. But in order to have more exact control of this most important condition, in a few promiscuously chosen instances (Exp. 17, 18, 19, and 21) the tissue included in the clamp has been examined microscopically for the presence in it of the auriculo-ventricular bundle of His. These histological examinations, which have been made by Retzer,[15] have invariably shown that the auriculo-ventricular bundle had been included in the clamp.

In most of the early experiments, heart-block was not obtained upon tightening the clamp or ligature. And in some of the recent experiments heart-block was not obtained at the first attempt. In these instances, the hook was withdrawn from the muscular septum and reinserted until a position was obtained at which tightening of the clamp produced heart-block. At the post-mortem examinations in these experiments the earlier positions that the clamp had occupied were sometimes marked by hæmorrhages into the tissue where it had been perforated by the point of the hook and where the tissue had been compressed in the effort to block the passage of the impulse. In all of these unsuccessful instances, the position which the clamp or ligature had occupied indicated that the auriculo-ventricular bundle had not been included in their grasp or had probably escaped compression for some other reason (Exp. 16).

[14] Since this paper was forwarded for publication this investigation has been completed and the results of it will appear in the *American Journal of Physiology*, 1906, No. 1.

[15] I wish here to express my thanks to Dr. Retzer for his kindness in making these valued and time-consuming examinations for me.

We have performed no experiments to determine the effect of destroying all connection between the auricles and ventricles excepting the region of the auriculo-ventricular bundle. This check was considered unnecessary because it has been so frequently made by other investigators. Krehl and Romberg investigated this problem directly, and as a consequence of *negative* results they have concluded that the impulse which normally causes the ventricles to contract passes through the auriculo-ventricular bundle of His. More recently Hering [16] has cut all of the auricular tissue, excepting the septum, away from the ventricles in the artificially perfused heart. No disturbance in sequence resulted. Furthermore, both Wooldridge [17] and Tigerstedt [18] state that in order to obtain heart-block it is necessary to destroy completely the functional continuity between auricles and ventricles. It can be easily understood that in experiments like those of Wooldridge and of Tigerstedt, in which the auriculo-ventricular junction is tied off *en masse*, or in which an attempt is made actually to cut across this region, the auriculo-ventricular bundle, owing to its protected position, would be apt to escape destruction. The evidence is therefore complete that the impulse which normally causes the ventricles to contract passes through the region of the auriculo-ventricular bundle.[19]

[16] Hering, *Zent. für Phys.*, 1903, xvii, 1.

[17] Wooldridge, *Arch. für Phys.*, 1883, 522.

[18] Tigerstedt, *Arch. für Phys.*, 1884, 497.

[19] The observations of Lomakina (*Zeit. für Biol.*, 1900, xxxix, 385) alone differ from those of other investigators. These are some of her conclusions: "Isolirte Unterbindungen oder Durchhitzungen solchen [Verbindungs] Fasern [nerves] stören die coordination zwischen Vorhöfen und Kammern, oder zwischen den beiden Kammern." "Nicht nur trennende Ligaturen zwischen Vorhöfen und Kammern unterbrechen den functioneller Zusammenhang, sondern auch Unterbindungen von oberen Theilen der Vörhofe und sogar besonders Schnurungen um die grossen Herzgefässe oberhalb des Herzens."

The marked discrepancies between these results and those of all other investigators who have studied this problem are perfectly obvious. Furthermore, the results obtained by Lomakina in presumably the same procedure differ so widely in the different experiments that we feel certain that faulty technique alone will account for them.

ADDENDUM.

In an experiment which has recently been performed by the author in collaboration with A. D. Hirschfelder, a 1 : 1 rhythm was changed into a 2 : 1

THEORETICAL CONSIDERATIONS.

Is the Functional Connection between Auricles and Ventricles of the Mammalian Heart Muscular or Nervous?—Histological investigations have shown that the only demonstrable muscular connection between the auricles and ventricles of the mammalian heart consists of the auriculo-ventricular bundle of His. The experiments herein reported, as well as those of others, have shown conclusively that damage to the tissue in the region in which this bundle lies alone will obstruct the passage of impulses from auricles to ventricles. These facts certainly afford logical evidence that in the case of the mammalian heart the impulse which normally causes the ventricles to contract is conducted through muscular tissue. In order to make this evidence absolutely conclusive it would be necessary to show that the tissue which has been included in the grasp of the clamp in successful experiments contains no nerve fibers. Naturally, this is not the case. There will undoubtedly be found in this tissue the nerve fibers which have been seen to ramify over the cells of heart muscle everywhere,[20] and nerve fibers which lie under the epicardium. But it certainly is improbable that such an important function as the conduction of the normal cardiac impulse is performed by scattered nerve fibers located in a limited region. If, as has been shown, the function of conduction is localized in a very limited region it seems reasonable to demand that a definite structure, whose function it is to conduct, be found in this region. If the conduction is through nerve there should

rhythm by pouring warm salt solution over the exposed auricles; and the 2 : 1 was converted into a 1 : 1 rhythm by pouring cold salt solution over the auricles. In the former procedure the auricular rate was increased, in the latter it was diminished. In addition we have shown that the phenomenon of stoppage of the ventricles may be obtained after the rhythm has been 2 : 1 for a long period of time, and that stoppage may occur in the fully atropinized heart. And finally, in this experiment the animal would almost certainly have died as a result of stoppage of the ventricles had not the ventricles been made to beat by artificial stimulation.

[20] See Berkley, *Johns Hopkins Hospital Reports*, 1895, iv, 748. Nerve fibers ramifying through the auriculo-ventricular bundle have since been found by Tawara, *Zent. für Phys.*, 1905, xix, 298.

be found in the region through which the impulse is conducted a definite nerve trunk or a well-developed nerve plexus. Retzer has made a careful examination of serial sections of the tissue included in the clamp in four successful experiments (Exp. 17, 18, 19, and 21) of this research. He informs me that the tissue contained no nerve trunks. And His states that no nerves are to be found in this region.[21] Therefore we maintain with Hering, that until it can be shown that the impulse which normally causes the ventricles to contract is conducted through the scattered nerve fibers which lie in the region of the auriculo-ventricular bundle, it must be admitted that the function of conduction between auricles and ventricles of the heart of the dog is performed by the muscular tissue of the auriculo-ventricular bundle.

This mode of conduction of the cardiac impulse may not be the same in all species of animals. It is undoubtedly the same in the case of the rabbit[22] and of man as it is in the dog. But Carlson[23] has obtained what appears to be convincing evidence that in the heart of limulus the conduction is through nerve. We do not, however, believe that Carlson is justified in inferring from his experiments that conduction in the vertebrate heart is the same as in the crustacean heart. To justify such an assumption it must first be shown that the physiological properties ot the heart muscle of limulus are the same as those of the heart muscle of mammals. If we may be justified in assuming that the physiological properties of the heart muscle of the American lobster are the same as those of the heart muscle of limulus, then it was first shown by Hunt, Bookman, and Tierney[24] that the crustacean heart muscle has none of the characteristic properties of vertebrate heart muscle excepting, perhaps, very limited spontaneous rhythmicity. Indeed, in its physiological characteristics, the heart muscle of crustaceans resembles skeletal muscle much more closely than it does vertebrate heart muscle. Thus it has

[21] "Nerven verlaufen in diesem Bündel nicht."

[22] His, loc. cit.

[23] Carlson, *American Jour. of Phys.*, 1905, xiii, 217. Here references to earlier papers by the same author will be found.

[24] Hunt, Bookman, and Tierney, *Zent. für Phys.*, 1897, xi, 274.

a very short latent period, it has no refractory period, its contractions may be summated and therefore tetanus may be obtained, and it does not obey the "all or none" law. In part, the observations of Hunt and his co-workers have been confirmed by Carlson[25] in the case of limulus. But Carlson seems to think it unnecessary to bring these facts into connection with the curious reactions of the heart of limulus to stimulation of extrinsic nerves. To one who has never worked with the invertebrate heart it does not seem at all surprising to find that a heart whose muscle has most of the properties of skeletal muscle, is governed more after the fashion of such a muscle than of the heart muscle of vertebrates.[26]

On the Cause of the Intersystolic Pause.—Hering[27] has suggested that the presence in the mammalian heart of a long, narrow, muscular bundle through which the impulse must pass on its way from the auricles to the ventricles explains very satisfactorily the delay between the beginning of auricular systole and of ventricular systole. This delay, which is so important to the proper functioning of the heart, was explained by Gaskell[28] in a similar way for the cold-blooded heart. Gaskell showed that to produce a block to the passage of the impulse in the cold-blooded heart it is only necessary to narrow down, by section, the muscle connecting the arterial with the venous ends of the heart. Furthermore, he found in the auriculo-ventricular junction of the tortoise heart circular strands of muscle more embryonic in structure than the adjacent musculature. These, he believes, conduct the impulse from auricles to ventricles. The slowness of the passage of the impulse he believes is due in part to the embryonic structure of the connection and in part to the indirectness of its course. It is interesting to note that these observations of Gaskell can be applied without any essential modification to the conditions that obtain in the mammalian heart. For the auriculo-ventricular bundle of His lies very

[25] Carlson, *American Jour. of Phys.*, 1905, xii, 471.
[26] See Howell, *Text-book of Physiology*, 1905, 498.
[27] Hering, *Pflüger's Archiv*, 1905, lvii, 104.
[28] Gaskell, *Jour. of Phys.*, 1883, iv, 70.

largely in the plane of the auriculo-ventricular junction and, according to Retzer,[29] its fibers have not the same staining properties as have those of the rest of the heart muscle.

In the case of the mammalian heart it cannot be proved that the length of the intersystolic pause depends upon the length and physiological properties of the auriculo-ventricular bundle. But it can and has been shown that slight compression of this bundle will distinctly delay the passage of the impulse from auricles to ventricles. The methods employed for recording the movements of the auricles and ventricles in this research were not sufficiently accurate to permit of exact measurement of the length of this delay. But they were sufficiently accurate to warrant the statement that the change in the rate of conduction which results when His's bundle is compressed is much greater than could possibly occur if the impulse were conducted through nerve fibers.[30]

The Action of the Vagi on the Ventricles.—One of the most constant results obtained while clamping the auriculo-ventricular bundle is the simultaneous appearance of complete heart-block and the complete, or almost complete, disappearance of the vagus control over the ventricles. This result may be interpreted in two ways:

(1) The vagi do not act directly upon the ventricles: stoppage of the ventricles when the vagus is stimulated is a consequence of the stoppage of the auricles, i. e. (and this amounts to the same thing), it is the consequence of the falling out of stimuli which normally cause the ventricles to contract.

(2) The vagi exert a positive influence upon the ventricles through the medium of the auriculo-ventricular bundle; clamping this bundle blocks the passage of this influence.

No conclusive evidence in the support of one or the other of these ideas has been obtained. But the facts (*a*) that the

[29] Retzer, verbal communication. It has since been found by Tawara (l. c.) that the fibers of the auriculo-ventricular bundle belong to the Purkinje system.

[30] We know of no research in which the effect of compression on the rate of transmission of the nervous impulse has been studied. Our statement is based upon the effect on nerve of other insults, for example, cold (Helmholtz, *Müller's Archiv*, 1850, 276).

ventricles usually continue to beat with their normal inherent rhythm at the moment complete heart-block is established, whereas (*b*) they stop beating when the auricles are inhibited, indicate that stoppage of the ventricles when the vagus is stimulated is the result of the arrival in the ventricles of a positive influence during inhibition.[31]

It might in addition be urged that when the block is complete the ventricles beat so slowly that, to use the suggestion of Gaskell,[32] anabolism is always at its maximum. Therefore stimulation of the vagus will produce no further anabolism and consequently the ventricles cannot be stopped. But the facts do not support this idea. When the rhythm is 2 : 1 or 3 : 1, the ventricles may be inhibited in most instances as readily as when the rhythm is normal. When the block is complete the rate of the ventricles may not be much slower than when the rhythm was 2 : 1 or 3 : 1, but now the ventricles cannot be inhibited by stimulation of the vagus.

But it will be recalled that, at the moment complete block is established, a very marked slowing of the ventricular rate may occur. If this marked slowing of the ventricular rate is the result of the sudden withdrawal of the stimuli of auricular origin, then it might be possible to ascribe stoppage of the ventricles when the vagi are stimulated to an absence of stimulation. The fact that stoppage of the ventricles does not always occur when the block becomes complete does not constitute an insuperable obstacle to the acceptance of this idea. For it is possible that when the block is slowly established time is allowed for something to occur which permits the ventricles to assume their maximum inherent rate at once. But, on the other hand, in some experiments in which complete block has been suddenly established there was no marked preliminary slowing of the ventricular rate. Still it is possible that the great variability in the reaction of hearts to stimulation of the vagus may account for such exceptions.

[31] Tigerstedt, *Physiologie des Kreislaufes*, 1893, 250.

[32] Gaskell, *Philosophical Transactions of the Royal Society of London*, 1882, pt. ii, 1029.

The fact that during complete block stimulation of the vagus may sometimes slow the ventricular rate very slightly and cause a slight fall of the blood-pressure may be interpreted in two ways. (1) A block which will effectually prevent the passage of the normal stimulus may not effectually prevent the passage of an inhibitory influence. (2) Inhibitory influences may reach the ventricles by other ways than through the auriculo-ventricular bundle. The vagus effect which has been observed during complete block has, however, been so very slight that it can be of but very little significance.[33]

It will therefore be seen that the problem of the mode of action of the vagi upon the ventricles is intimately associated with the phenomena upon which is dependent the stoppage of the ventricles occurring at the moment when complete block is established. Although some effort has been made to solve the latter problem, a sufficient number of facts has not yet been collected to justify a final statement with regard to it.

CONCLUSIONS OF PART II.

The more important results of the second part of this paper may be summarized as follows:

(1) In the dog the impulse which normally causes the ventricles to contract is conducted through the auriculo-ventricular bundle of His.

(2) By compression of this bundle all stages of heart-block may be obtained. These include:

(*a*) An increase of the intersystolic pause;
(*b*) An occasional ventricular silence;
(*c*) Regularly recurring ventricular silences—e.g., one silence in ten, nine, eight, seven, six, five, four, three, and two auricular beats;
(*d*) A 2 : 1 rhythm;
(*e*) A 3 : 1 rhythm;
(*f*) Complete heart-block.

[33] It is indeed possible that these effects may be wholly or in part due to the inhibition of the auricles.

(3) As a rule the ventricles take on a constant slow rate at the moment complete heart-block is established. Occasionally, usually when the block becomes complete suddenly, there results a marked preliminary slowing of the ventricular rate. The auricular rate does not change.

(4) When the block is complete, stimulation of the vagus nerve has no, or but a minimal, effect upon the rate and force of the ventricular beats, whereas the auricles still react normally.

(5) When the block is complete, stimulation of the accelerator nerve increases the rate both of the auricles and the ventricles.

(6) When the block is complete, the rate of ventricular beats may not be materially affected by variations in the general blood-pressure, nor by asphyxia, nor by interference with the coronary circulation.

PART III.

THE RELATION OF HEART-BLOCK TO STOKES-ADAMS DISEASE.

THE ANALOGIES BETWEEN HEART-BLOCK IN MAN AND HEART-BLOCK IN THE DOG.

A mere perusal of Parts I and II of this paper should suffice to impress upon the reader the close relation which exists between the results obtained from a study of a case of heart-block in man and heart-block in the dog. These relations are so close that there is no reason to doubt that the condition in man is caused by the same factor which produces the condition in the dog. That heart-block in man is the result of a diminution in the conductivity of the auriculo-ventricular bundle of His, is borne out not alone by the fact that a condition of heart-block exists, but also by the facts that the vagus has lost its influence over the ventricles, that presumably the accelerator nerves still preserve their influence over the ventricles, and that the ventricles may stop beating for long periods of time while the auricles continue to beat without interruption.

It is true that in man marked slowing of the ventricular rate

usually occurs at times when the block is complete, whereas in the dog we have not been able to obtain a marked reduction of the ventricular rate after complete heart-block has been established. But we believe that this difficulty is merely the result of the crudeness of experimental methods as compared with the subtle inroads of pathological processes. For example, in animal experiments it is not possible to maintain a partial block for any considerable length of time. Usually within a very few minutes the partial block will disappear either in one direction or the other. But in man the condition of partial block may be maintained for days at a time. And when it is recalled that the cause of the slowing of the ventricles both in man and in the dog is obscure, the mere fact that in both marked slowing of the ventricles alone may occur should suffice to establish the identity of the lesion. We therefore state without hesitation that in our case of Stokes-Adams disease the lesion upon which the symptoms depended involved the auriculo-ventricular bundle of His.

WILL A LESION IN THE VICINITY OF THE AURICULO-VENTRICULAR BUNDLE ACCOUNT FOR ALL OF THE TYPICAL SYMPTOMS OF STOKES-ADAMS DISEASE ?

The cardinal symptoms of Stokes-Adams disease, all of which were noted by Stokes,[34] are:

(1) A slow pulse sometimes associated with pulsations in the veins of the neck which may be more than twice as frequent as the manifest beats of the ventricles.[35]

(2) Syncopal attacks which may be epileptiform or apoplectiform in character and in which the pulse-rate is unusually slow.

With regard to the former symptom, it is perfectly obvious

[34] Stokes, *Dublin Quart. Jour. of Med. Sciences*, 1846, ii, 73.

[35] Stokes makes the significant statement that "the number of reflex pulsations (in the veins) is difficult to be established, but they are more than double the number of the manifest ventricular contractions." As this statement precedes the publication of the epoch-making experiments of Stannius, it does not seem probable that Stokes meant to imply that the auricles and ventricles were beating independently of one another.

from what has already been said that in heart-block the pulse is slow and that the auricles will usually develop venous pulsations of more than twice the frequency of the apex beats.

And with regard to the latter, it has also been shown that in heart-block there may be periods during which the pulse-rate is unusually slow even for heart-block. Evidence has also been presented that the syncopal attacks are certainly the result of slowing of the ventricular rate. And Webster [36] first forcibly called attention to the fact that the epileptiform seizures of Stokes-Adams disease may be caused by the anæmia of the brain resulting from failure of the heart to supply a sufficient quantity of blood. For Kussmaul and Tenner,[37] and many others since them, have shown that "the convulsions appearing in profuse hæmorrhage of warm-blooded animals (including man) resemble those observed in epilepsy," and that "when the brain is suddenly deprived of its red blood, convulsions ensue of the same description as those occurring subsequent to ligature of the great arteries of the neck."

The apoplectiform attacks might also be accounted for in much the same way. Kussmaul and Tenner have shown that "venous congestion of the brain, as well as arterio-venous congestion, brings about conditions which belong more to those of apoplexy than to those of epilepsy, and are characterized by paralysis of the glottis, together with a slower respiration and slight spasmodic symptoms." This state of affairs might obtain in those syncopal attacks in which the slowing of the ventricles is not sufficient to cause convulsions, but will just cause complete unconsciousness. In such a case the venous congestion would be marked, and the fall of the arterial pressure might not be extreme.

It is therefore evident that all of the cardinal symptoms of Stokes-Adams disease may be duplicated by heart-block and its attendant phenomena.

[36] Webster, *Glasgow Hospital Reports*, 1900, iii, 413. See also, Bristowe, *Lancet*, 1885, i, 447; and 1894, ii, 671; and Gibbings, *Lancet*, 1885, i, 288.

[37] Kussmaul and Tenner, *Epileptiform Convulsions from Hæmorrhage*, New Sydenham Society, 1859.

IS HEART-BLOCK ALWAYS THE CAUSE OF STOKES-ADAMS DISEASE?

It has been shown that all of the symptoms of Stokes-Adams disease may be caused by a lesion which involves either directly or indirectly the auriculo-ventricular bundle of His—i. e., by heart-block. It has also been shown that in the two typical cases of Stokes-Adams disease herein described heart-block did exist. The question then arises, Is heart-block the pathogenetic factor of all cases of Stokes-Adams disease?

With the object of answering this question it was at first intended to examine the histories of all cases of Stokes-Adams disease that have been put on record. But it was soon found that this method of deciding the matter would be impracticable. For the number of cases that have been described is enormous,—very much larger than the recent collections would lead one to suppose.[38] And, furthermore, in the vast majority of instances the data given do not suffice to rule out heart-block, much less to prove that heart-block existed. This much may, however, be said: in not a single instance among the many cases that have been examined, has the description of a case of typical Stokes-Adams disease precluded the existence of heart-block. Indeed, it may be stated positively that in many instances the data sufficed for the diagnosis of heart-block.[39]

But if it is justifiable to choose from all of the cases that have been described only those that have been studied by methods of sufficient accuracy to permit of exact diagnosis, then it may be

[38] Collective references to cases of slow pulse and cases of Stokes-Adams disease have been made by Prentiss, *Trans. of the Assn. of American Phys.*, 1889, iv, 120; Webster, loc. cit.; Edes, *Trans. of the Assn. of American Phys.*, 1901, xvi, 521; Jaquet, *Deutsches Arch. für klin. Med.*, 1902, lxxii, 77. Osler (*Lancet*, 1903, ii, 516) has described twelve previously unreported cases of Stokes-Adams disease.

[39] The criteria employed in reaching this decision are laid down in the section on diagnosis. As an example of the difficulties in the way of using this method of analyzing cases, the following may be mentioned. A case of Stokes-Adams disease was described by Figuet (Thesis, Lyon, 1882) as a typical instance of "rythmé couplé." About one year later, Chauveau (the full report of this case appeared in *Rev. de méd.*, 1885, v, 161) demonstrated that the case was one of heart-block. This, by the way, is the first demonstrated instance of heart-block in man.

stated that all such cases of Stokes-Adams disease have proved to be instances of heart-block. It is true that in a few of these instances the exact condition was not recognized by the authors, indeed, one of the authors states that in his case the phenomena were not caused by heart-block. But in each sase, had the proper interpretation of the tracings been made, the diagnosis of heart-block would have been reached.

The cases which have been used as a basis for the statements of the foregoing paragraph are collected in the accompanying table.

TABLE XVII.

CASES OF STOKES-ADAMS DISEASE WHICH ARE ACCOMPANIED BY DATA SUFFICIENT FOR AN EXACT DIAGNOSIS OF THE CARDIAC CONDITION. (INCLUDING TWO CASES OF HEART-BLOCK WITHOUT SYNCOPAL ATTACKS.)

Author.	Where Described.	Diagnosis of Author.	Etiology.	Remarks.
1. Chauveau.	*Rev. d. méd.*, 1885, v, 161.	Heart-block.	—	Tracings.
2. Vaquez and Bureau.	*C. r. Soc. d. Biol.*, 1893, v, 169.	"	Abdominal traumatism.	Tracings. No syncopal attacks.
3. Vaquez and Bureau.	*C. r. Soc. d. Biol.*, 1893, v, 169.	"	—	Tracings.
4. His, Jr.	*Deutsches Arch. f. klin. Med.*, 1899, lxiv, 316.	"	—	Tracings.
5. Hoffmann.	*Zeit. f. klin. Med.*, 1900, xli, 357.	"	Anæmia (?)	Tracings and X-rays.
6. Webster.	*Glasgow Hosp. Rep.*, 1900, iii, 413.	No diag.	—	Tracings of radial pulse only, but these show two perfect and independent rhythms.
7. Wenckebach. (2 cases ?)	*Die Arhythmie*, 1903.	Heart-block.	Arterio-sclerosis (?)	Tracings.
8. Jaquet.	*Deutsches Arch. f. klin. Med.*, 1902, lxxii, 77.	Extra-systoles.	Syphilis.	Tracings misinterpreted: two perfect and independent rhythms in cardiogram.
9. Lichtheim.	*Deutsche med. Woch.*, 1902, xxviii, 69.	Heart-block.	Arterio-sclerosis (?)	Tracings—3 : 1 rhythm.
10. Mackenzie.	*Brit. Med. Jour.*, 1902, ii, 1411.	"	Influenza.	Tracings. No syncopal attacks: but condition acute, and patient constantly in bed.
11. Gerhardt.	*Schmiedeberg's Arch.*, 1904, li, 11.	"	—	Tracings.
12. Schmidt.	Quoted by Finckelnburg.	"	(?)	X-rays.
13. Finckelnburg	*Deutsches Arch. f. klin. Med.*, 1905, lxxxii, 586.	"	—	Tracings.

THE DIAGNOSIS OF HEART-BLOCK—STOKES-ADAMS DISEASE.

Undoubtedly every stage of interference to the passage from auricles to ventricles of the impulse which normally causes the

heart to beat should be included under the head of heart-block. However, that stage in which the block just suffices to increase the length of the intersystolic period would not give rise to symptoms, and consequently it would be but rarely found, merely because it would be but rarely looked for. But any stage beyond this would be characterized by the existence of perfectly regular auricular beats, some of which would not be followed by ventricular beats. In the early stages there would be occasional *ventricular silences*. Later these might recur regularly. Then every other auricular impulse might fail to stimulate the ventricles, and so on until none of the auricular impulses would arrive in the ventricles. Then the auricles and ventricles would beat totally independently of one another, the ventricles much more slowly than the auricles, but each with perfectly regular rhythms, barring, of course, the existence of those extraneous circumstances which in other states give rise to cardiac arhythmia.

In order to make the diagnosis of heart-block it is, therefore, necessary to show that the ventricles more or less regularly fail to respond to one or more of the regularly recurring auricular impulses.

Naturally, the most certain method of arriving at a correct diagnosis consists in making tracings of one or of all of the following: apex beat, jugular pulse, and arterial pulse. When the case is perfectly clear cut, and the signs distinct, it is conceivable that any one of the above tracings might suffice for the diagnosis. For the marks of both auricular and ventricular activities may, as in the case of our patient, be evident on all of them. If there can be distinguished in any one of these tracings the marks of two perfectly regular rhythms the diagnosis of heart-block may be made with reasonable certainty.

We can at present recall but two possible, though rare, sources of error. (1) In the form of arhythmia to which the name alternating pulse has been given, two perfectly regular rhythms are stated to occur: a strong heart-beat is regularly followed by a weak heart-beat, and these may be equally spaced. But in this condition the signs which are dependent upon the feebler

beats are much too distinct to be confused with the signs produced by the beats of the auricles alone. (2) Extra-systoles of ventricular origin might rarely simulate heart-block. We have recently had the opportunity of studying a case of this kind. The tracings obtained are shown in Fig. 27. At the time this tracing was made a ventricular extra-systole occurred after every fourth normal systole. The signs of the extra-systoles are perfectly clear in the cardiogram. The cardiogram of a case of this kind could, therefore, never give rise to ambiguity. But at first sight the main waves on the jugular pulse appear to be spaced by equal distances. However, measurement shows that this is not the case; the larger waves occur somewhat earlier than the smaller waves. The larger waves are produced by auricular contractions, the stimuli for which are derived from the retrograde impulses generated by the ventricular extra-systoles. It is, of course, possible that the retrograde impulses might arrive in the auricles at such a time as not to interfere with the normal auricular rhythm. In such a case the auricular rhythm would be perfect, while in the peripheral arteries there might be no wave to correspond with one of the auricular beats. Therefore, in a case of this kind, if tracings of the venous and arterial pulses alone were made, there might be some difficulty in arriving at a diagnosis; but this difficulty would disappear upon comparing the arterial and venous tracings with the cardiogram. Therefore ventricular extra-systoles need not give rise to confusion in the diagnosis of heart-block.

But in most instances it should be possible to arrive at a diagnosis of heart-block with the aid of the usual clinical methods alone. Thus, after the author had become thoroughly familiar with the condition, there was usually but little difficulty in determining the auricular and ventricular rates by inspection of the veins of the neck. And we believe that in most cases when the block is complete or partial, with at least a 2 : 1 rhythm, it will be found possible to distinguish the two regular rhythms in the veins of the neck—rhythms independent of one another in complete block or with a definite ratio in partial block. Upon inspection of the apex-beat the same double rhythm might be

made out in favorable cases, but the movements produced by the beats of the auricles would, at best, be very indistinct. Upon auscultation at the apex the same double rhythm might be discovered: the sounds associated with the movements of the heart synchronous with the pulse-waves in the peripheral arteries would be loud and distinct; between these might be heard the very feeble and distant sounds produced by the beats of the auricles.

So far as we know, the only condition that might be confused with this is one to which we have already referred, namely, extra-systoles of ventricular origin. But Hochhaus and Quincke[40] have shown, and we have recently had the opportunity of confirming this in a single case, that the apex-beat and heart sounds associated with an extra-systole are fully as distinct as those of a normal systole.[41]

The results which we have obtained in our animal experiments and during the recovery of our case of Stokes-Adams disease seem to indicate that an increase in the length of the intersystolic period, together with an occasional ventricular silence, will probably be found to be the first sign of beginning Stokes-Adams disease. These disturbances could be determined only by means of carefully made tracings. The methods of interpreting such tracings have been so fully considered by Wenckebach[42] and by Mackenzie[43] that nothing can be added to their descriptions.

Finally, we believe that the Stokes-Adams syndrome of slow pulse with syncopal attacks, during which the pulse is unusually slow, will in itself be found to be pathognomonic of heart-block. At least no other pathological entity can be conceived which could develop just this combination of symptoms. But cases of

[40] Hochhaus and Quincke, *Deutsches Arch. für klin. Med.*, 1894, liii, 415.

[41] Many cases of slow pulse have been described as instances of so-called hemisystole. But we maintain with Hering (*Deutsche med. Woch.*, 1903, xxxii, 281) that hemisystole is not a well-characterized physiological condition and that there is no demonstrated case of hemisystole on record. The first supposed instance described, that of Leyden (*Virchow's Archiv*, 1868, xliv, 365), is clearly one of ventricular extra-systole.

[42] Wenckebach, *Die Arhythmie*, etc., 1903.

[43] Mackenzie, *British Med. Jour.*, 1905.

heart-block without syncopal attacks have been described, and the question therefore arises—Should a distinction be made between heart-block without syncopal attacks and heart-block with syncopal attacks? It is, of course, possible that the lesion which causes the heart-block might be different in the two groups. But clinical records indicate that they merely represent two stages of the same disease. Cases have been described in which the syncopal attacks made their first appearance long after the bradycardia. In our second case it required about one year for the full development of the Stokes-Adams syndrome. And it is well known that the frequency of the syncopal attacks increases as the end approaches. We therefore believe it will be shown that the distinction between heart-block with and without syncopal attacks is an arbitrary one.

THE ÆTIOLOGY OF STOKES-ADAMS DISEASE.

Although many cases of Stokes-Adams disease have come to autopsy, and although many lesions have been discovered in such cases, these lesions have been very variable and in practically no case has it been possible to show any direct relation between the lesion and the symptoms presumably caused by it. Among other things, there have been described sclerosis of the cerebral arteries and of the coronary arteries, fatty degeneration of the heart, tumors pressing upon the vagi, fracture of the odontoid process, with enlargement of the middle cervical ganglion, etc. In some cases nothing has been found. From the evidence which has been presented in this paper, the conclusion is warranted that a lesion which causes heart-block must be located in or near the auriculo-ventricular bundle: in it, if it is a degenerative lesion; near it, if it acts by compressing the bundle. We have been able to find but one case of Stokes-Adams disease in which there existed a lesion that might have caused heart-block by compressing the auriculo-ventricular bundle. Sendler [44]

[44] Sendler, *Cent. für klin. Med.*, 1892, p. 642. Two cases of Stokes-Adams disease have recently been described in which it is probable that a lesion in the auriculo-ventricular bundle was the cause of the trouble: Stengel, *Amer. Jour. of the Med. Sciences*, 1905, cxxx, 1083, and Hay, *Brit. Med. Jour.*, 1905, ii, 1034.

describes a case with a pulse of 40 and typical syncopal attacks, in which there was found at autopsy a cartilaginous mural tumor the size of a walnut. It was situated about one centimeter below the ostium of the pulmonary artery, but did not touch the valves. No other lesion has been found here, probably because it has never been carefully looked for. As a discussion of this matter must therefore be hypothetical and unsatisfactory, we shall refer to but two of the possibilities, one of which is of more than passing interest.

It may have been a coincidence that both of the cases that have come under our observation have given clear histories of syphilis. It may also have been a coincidence that one of the cases which has been under observation for a sufficiently long interval of time has practically recovered under treatment with potassium iodide, recommended by Dr. Osler. But when it is recalled that Jaquet's patient, who also gave a syphilitic history, was distinctly benefited while under an antisyphilitic treatment,[45] it seems to be more than likely that syphilis may be one of the ætiological factors of Stokes-Adams disease. But if syphilis causes heart-block, it certainly is not the only cause; at least it does not seem to be mentioned more frequently in the histories of cases of Stokes-Adams disease than in the histories of other diseases.

The second possibility is one for which no basis has been obtained by observation. The close relation which the mesial leaflet of the tricuspid valve bears to the auriculo-ventricular bundle of His makes it appear as probable that disease of this leaflet might, by putting His's bundle under tension, interpose a block to the passage of normal impulses from auricles to ventricles. At any rate, the condition of this leaflet should be carefully determined in all cases of Stokes-Adams disease that come to autopsy.

THE TREATMENT OF STOKES-ADAMS DISEASE.

It is not the purpose of the author in writing this section to detail the symptomatic treatment of Stokes-Adams disease. It

[45] Jaquet, loc. cit.

is intended to refer here to but two phases of the subject which have been encountered during the course of this investigation.

In the first place, the apparent recovery of one of the cases under treatment with potassium iodide should here be recalled. For this result makes it desirable to emphasize the importance of determining the presence of, and of treating, syphilis in cases of Stokes-Adams disease. But the fact that Hoffmann saw a case recover under repeated administrations of oxygen makes it additionally desirable to determine in these cases of recovery the parts played by treatment and the natural defences of the individual.

The second point to which reference should be made is the effect which the administration of atropine had in temporarily stopping the attacks of syncope. Although the results of this research justify the statement that atropine will not increase materially the slow pulse-rate of heart-block, still it is possible that in mild cases atropine, by steadying the pulse-rate, might tend to ward off attacks of syncope.

CONCLUSIONS.

The facts collected in the third part of this paper justify the following conclusions:

(1) All of the cardinal symptoms of Stokes-Adams disease may be duplicated by heart-block resulting from a lesion in or near the auriculo-ventricular bundle of His, and by this alone.

(2) No typical case of Stokes-Adams disease has been described in which heart-block might not have been the cause of the trouble.

(3) It can be shown that all cases of Stokes-Adams disease which have been studied by sufficiently accurate methods were cases of heart-block.

(4) It would appear that heart-block without and with syncopal attacks are two stages of the same disease process.

APPENDIX.

PROTOCOLS OF EXPERIMENTS OF PART II.

Series A.—Experiments in which Ligatures Were Used.

Exp. 1. Feb. 1, 1905. As soon as ligature was passed and before it was tied the whole heart went into fibrillary contractions from which it did not recover.

Autopsy.—Ligature entered heart anterior to membranous septum, passed directly into the right ventricle, and out of it posterior to the membranous septum.

Exp. 2. Feb. 7, 1905. Ligature passed and tied without any effect on the heart.

Autopsy.—Ligature entered right ventricle anterior to membranous septum, passed upward into the right auricle and emerged from it about 1 cm. from the root of the aorta.

Exp. 3. Feb. 9, 1905. Before passing ligature obtained partial block with 2:1 rhythm upon stimulating vagus with coil at fifteen; with the coil at thirteen complete block was obtained. The first ligature was passed without effect on the heart. The second ligature was entered at about the same place as the first, but was brought out somewhat farther posteriorly. As soon as it was passed and before it was tied the auricles went into fibrillary contractions. The ventricles did not stop, but continued beating with a very slow rate. During this period, which lasted about ten minutes, the ventricles could not be inhibited even with the coil at zero. Then the auricles began to beat, but with a rate very much faster than that of the ventricles and the rhythms were independent of one another, auricle beating about three times to ventricle once. At this time stimulation of the vagus with the coil at thirteen gave complete inhibition of the auricles, but had absolutely no effect upon the ventricles. Strongest stimulation of the vagus (coil at zero) had no influence upon the ventricles.

This condition lasted for about a half-hour when the complete block changed into a partial block with a 3 : 1 rhythm. Now stimulation of the vagus with the coil at fifteen gave complete inhibition of the whole heart which could be maintained for some time. The partial block gradually disappeared through a 2 : 1 rhythm. When the normal rhythm had returned it was not possible to produce a partial block by stimulation of the vagus with any strength of stimulus. Now the second ligature, which had simply been passed, was tied, but without influence on the condition of the heart.

Autopsy. On opening the heart both ligatures slipped out, therefore the positions they had occupied could not be determined.

Exp. 4–7. All failed. In two the heart was torn while making the preliminary dissection, and hæmorrhage prevented further work. In one the only fine needle possessed broke. In another the ligature was passed and tied with negative results. Autopsy showed that the ligature had not passed into ventricular muscle at all; it had passed through auricular tissue only.

Series B.—Experiments with Vise Clamp.

Exp. 8. Feb. 15, 1905. Clamp was put in place without difficulty and without disturbing normal sequence of heart-beat. Tracings of auricular and ventricular movements were made with the myocardiograph of Roy and Adami; there was no time record, hence the results can be expressed only relatively.

(A) Stimulation of vagus: complete inhibition of whole heart.

(B) Clamp tightened until complete block came on. The marks of the ventricular contractions can be seen on auricular tracing. From this it may be seen that the ventricular systole falls in any phase of the auricular cycle. In the course of 47 auricular contractions the ventricles contract 7 times.

(C) Now stimulation of vagus gave complete inhibition of the auricles, but the ventricles continued to beat. Before stimulation of vagus one ventricular systole occurred regularly in 7 mm. of tracing. During vagus stimulation one ventricular systole occurred in 9 mm.; after inhibition one ventricular systole in 8 mm.

(D) Second stimulation of vagus. Complete inhibition of auricles. Ventricular rate increased: one ventricular systole in 3 mm. First ventricular cycle after inhibition 10 mm., then normal rate: one ventricular systole in 7 mm.

(E) Third stimulation of vagus. Complete inhibition of the auricles, ventricular rate increased more or less gradually, becoming one ventricular systole in 2.5 mm. After inhibition the distances between successive ventricular systoles = 10, 8, 7.5, 7, 7, 7.5, 7.7 mm.

(F) Unclamped: normal sequence returned.

The whole experiment was repeated with similar results.

Autopsy.—Posterior arm of clamp entered the left ventricle posterior to the membranous septum and through the auriculo-ventricular junction. It passed anteriorly, piercing one of the leaflets of the aortic valve. The anterior arm entered the right ventricle anterior to the membranous septum, about 3 mm. below the auriculo-ventricular junction and ran posteriorly in the cavity of the right ventricle.

Exp. 9. Feb. 17, 1905. While passing the clamp the auricles twice went into fibrillary contractions, but recovered. To make room for the myocardiograph the clamp was drawn to the left side under some tension. Without warning, the ventricles went into fibrillation, and the dog died.

Autopsy: Both arms of the clamp entered the heart at the auriculo-ventricular junction, the posterior one as in preceding experiment; the anterior one pierced the middle of the membranous septum.

Exp. 10. Feb. 21, 1905. Auricular contractions recorded by tambours, ventricular contractions and blood-pressure by mercury manometer connected with carotid artery. Time in seconds inscribed on base-line. Passed clamp with no effect. Clamped with no effect. However, stimulation of vagus was now associated with a remarkable phenomenon. The heart could be inhibited as easily as before clamping, but while the heart was recovering from inhibition, both the auricles and ventricles went into contractions which resembled fibrillation very closely. This was repeated several times.

Autopsy.—The anterior arm of clamp passed through the muscular septum anterior to the membranous septum. The posterior arm entered the aorta too high and at the posterior edge of the membranous septum.

TABLE XVIII.

Procedures and Remarks.		No. of Vs.	Duration in Sec.	V. Rate per Min.	No. of As.	Duration in Sec.	A. Rate per Min.	— V	Max. Pres.	Min. Pres.
Stim. of vagus, medium.	Before.	8	13.8	34.8	32	14.8	130	3.7	41	21.5
	During.	6	12+	30	0	—	—	—	34	17+
	After.	7	12.2	34.4	24	11.6	124	3.6	46	28
Same, stronger.	Before.	5	8.1	37	16	7.3	131	3.5	48	32.5
	During.	5	7.7	38.9	0	—	—	—	42	32.5
	After.	4	7	34.2	12	6.2	116	3.4	42.5	27
Same, weak.	Before.	5	8.4	35.7	17	8	127.5	3.6	42	28.5
(As. cannot be counted).	During.	6	10.2	35.3	0	—	—	—	36	25
	After.	3	5.1	35.3	—	—	—	—	40	27.5
Same, very strong.	Before.	5	8.6	34.9	22	10.6	124.5	3.6	42	32
(As. cannot be counted.)	During.	8	14.6	32.9	0	—	—	—	irreg.	
	After.	4	7.3	32.9	—	—	—	—	"	
Compression of abd. aorta.	Before.	5	9.2	32.6	18—	9.2	117	3.6	43	34
	During.	7	13.1	32.1	25+	13.1	115	3.6	53	41
	After	4	7.6	13.6	14.5	7.6	114.5	3.6	38.5	30
Same.	Before.	6	12.1	29.8	22	12.1	100	3.7	48	38
	During.	8	16.1	29.8	29.7	16.1	111	3.7	62	49
	After.	3	6	30	11	6	110	3.7	44	36
Compression of thor. aorta.	Before.	5	10.5	28.6	19.5	10.5	111.5	3.9	42.5	33
	During.	6	12.9	27.9	24	12.9	112	4	65	47
	After. {	5	11.2	26.8	21	11.2	112.5	4.2	39	31
	{	7	15.2	27.6	28	15.2	110.5	4	42	33
Stimulation of vagus, weak.	Before.	4	8.6	27.9	16	8.6	111.5	4	41	32
	During.	6	13.1	27.5	greatly reduced	—	—	—	38	32
	After.	4	8.8	27.3	15.5	8.8	106	3.9	39.5	31.5
Same, medium.	Before.	6	13.1	27.5	22	11.9	111	4	40.5	32
	During.	6	13.7	26.3	0	—	—	—	37	32
	After.	3	6.8	26.5	15	8.9	101	3.8	39	30.5
Same, strong.	Before.	5	11.2	26.8	17	9.4	108	4	38	30
	During.	5	11.5	26.1	—	—	—	—	irreg.	
(A. does not record.)										
No procedure.		6	21.1	17.1	—	—	—	—	22	16

Exp. 11. Feb. 23, 1905. While passing the second half of clamp the first half slipped out. The first half of the clamp was reinserted blindly through the hæmorrhage. When the clamp was tightened no block resulted.

Autopsy.—Both arms are above the auriculo-ventricular junction and embrace auricular tissue only.

Exp. 12. Feb. 24, 1905. I had some difficulty in adjusting the clamp; one arm slipped out of the heart. The hæmorrhage that resulted made it difficult to reinsert clamp. After readjustment, tightening of the clamp had no influence on sequence.

Autopsy.—The clamp embraced auricular muscle and the wall of the aorta only.

Exp. 13. Feb. 24, 1905. While introducing the clamp I got momentary heart-block, but tightening the clamp had no influence upon sequence nor upon the ease with which the whole heart could be inhibited.

Autopsy.—Both arms entered the heart above the line marking the auriculo-ventricular junction and embraced auricular tissue and aortic wall only.

Exp. 14. Feb. 25, 1905. Posterior arm of clamp inserted without mishap. At the instant the anterior arm was passed, and before tightening, complete heart-block came on and persisted throughout the experiment, three quarters of an hour. Auricular contractions were recorded with tambours; ventricular contractions and blood-pressure with mercury manometer in the carotid artery; time is marked in seconds on base-line.

Autopsy.—Posterior limb of clamp was in left ventricle some distance posterior to membranous septum. The anterior limb entered the left ventricle just below the aortic valves, perforated the middle of the membranous septum, and entered the right ventricle; turned posteriorly and entered the muscular septum about 2–3 mm. posterior to the posterior edge of the membranous septum, and the point lay in the left ventricle. Hence the portion of the muscular septum between the posterior edge of the membranous septum and a point 2–3 mm. posterior to this was gathered on the anterior limb of the clamp. Both coronary arteries were patent.

The analysis of the tracings is given in Table XVIII.

Series C.—Experiments with Fishhook Clamp.

Exp. 15. Feb. 28, 1905. Large dog. Clamp was passed and experiment begun at 4.30 P.M. and concluded at 6.00 P.M. Details of the experiment are given in Table XIX. At one time the clamp was left tightened for over 45 minutes, and upon loosening it, normal sequence returned. At the end of the experiment, while killing the animal by asphyxia and at a time when the animal was all but dead, the clamp was loosened and normal sequence returned and persisted until the heart stopped.

Autopsy.—Hook entered the aorta exactly at its root, at the upper and posterior edge of the membranous septum. It pierced the muscular septum about 3 mm. below, and posterior to, the posterior edge of the membranous septum.

Exp. 16. March 2, 1905. Small dog. Clamp adjusted on heart, but clamping produced no block.

Autopsy.—Clamp was placed about as in preceding experiment. Negative result was probably due to fault in clamp. A large space close in toward the hook could not be obliterated; important tissue might have been in that place. A new clamp was made in which there could be no dead space. This clamp was used in all subsequent experiments.

Exp. 17. March 3, 1905. Very large dog. While dissecting alongside of the aorta there was considerable hæmorrhage which was finally controlled. Clamp was introduced without producing block. Upon tightening the clamp complete block came on promptly, auricular to ventricular rate being about 4 : 1. While preparing to insert coronary sound the dog died.

TABLE XIX.

Procedures and Remarks.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec	A. Rate per Min.	$\frac{A}{V}$	Max. Pres.	Min. Pres.
Clamp open.										
Stim. of vagus, strong.	Before.	45	59.5	227	45	59.5	227	1	75	73
	During.	3	25.6	35.2	0	—	—	—	52	35
	After.	50	61.2	245	50	61.2	245	1	71	69
After clamping.										
Stim. of vagus, same.	Before.	20	86	69.8	62	86	216	3.1	59	49
	During.	13	58	67.2	0	—	—	—	48	38.5
	After.	20	78.6	76.3	61	78.6	233	3.05	55	47.5
After unclamping.										
Stim. of vagus, same.	Before.	37	49.6	224	37	49.6	224	1	74	72
	During.	2	30.5	20	0	—	—	—	—	21
	After.	36	47	230	36	47	230	1	65	63
Clamping and unclamping.										
Clamping (stopped as soon as block appeared).		43	60.5	213	43	60.5	213	1	69	67
The above passes immediately into:		14	39.2	107	28	39.2	214	2	63	60
The above is followed by, 1-3:1, 2-2:1, then:		7	28.4	74	21	28.4	222	3	58	52
Followed by 1-4:1(?), 2-3:1, then:		8	37.8	63.5	26.5	37.8	210	3.3	53	46
Unclamped completely,		9	38	71	27	38	213	3	56	50
Followed by 1-2:1, 1-3:1, 2-2:1, 1-3:1, then:		4	10.7	112	8	10.7	224	2	64	60
Then:		20	27.8	216	20	27.8	216	1	70	68
Clamped.										
Effect of asphyxia (stoppage of art. resp.).	Before.	10	43	69.8	30	43	209	3	59	53
Approaching highest pressure.		8	31	77.4	—	—	—	?	125	120
Art. resp. resumed.										
Pressure falling.		6	30.6	58	19 (?)	30.6	201	3.4 (?)		
Pressure constant.		12	58.8	61.2	46	58.8	235	3.8	86	78
Later.		11	59	55.9	45	59	229	4.1	94	85
Compression of abd. aorta.	Before.	10	59	50.8	43	59	219	4.3	66.5	59
	During.	9	54	50	40	54	220	4.4	85	76
Compression of abd. aorta.	Before.	10	66	45.5	46.5	66	211	4.6	68	58
	During.	10	68.2	44	48	68.2	211	4.8	95	83
	After.	10	66	45.5	45	66	205	4.5	50	40
Stim. of vagus: coil at 0.	Before.	10	67	44.3	45	67	201	4.5	58	49
	During.	5	47.5	31.6	0	—	—	—	46	35
	After.	10	59	51	45	59	229	4.5	56	48
Same.	Before.	10	68	44.1	47	68	207	4.7	61	51
	During.	5	48	31.2	0	—	—	—	47	36
	After.	10	59.6	50.3	45	59.6	226	4.5	56	47
Effect of cutting both vagi.	Before.	10	65.2	46	46	65.2	213	4.6	65	56
During (pres. at max.).		10	62.4	48.1	51	62.4	245	5.1	119	108
	Later.	10	67.5	44.4	54	67.5	240	5.4	99	90
After unclamping.		43	53.2	242	43	53.2	242	1	123	122
After clamping.										
Effect of asphyxia.	Before.	10	56	53.6	45	56	241	4.5	87	80
During (pres. rising).		10	59.2	50.7	48.5	59.2	246	4.8	147	137
During (pres. falling).		8	55	43.6	44	55	240	5.5	falling	
Art. resp. resumed (pres. almost at min.).		10	69	43.5	56	69	243	5.6	92	80
(Pres. rising subsequently.)		10	62.2	48.2	50	62.2	241	5	116	107
After cutting both splanchnics (long interval.)		5	77	19.5	?				25	17
After unclamping.		30	62.6	114	30 (?)	62.6	144	1	22	

Autopsy—Both coronaries patent. The hook had passed into the aorta, perforating the anterior edge of the posterior leaflet of the aortic valve; passed downward, entered the muscular septum just posterior to the membranous septum, and, running obliquely backwards and downwards, entered the right ventricle at a place behind the center of the mesial leaflet of the tricuspid valve.

The bit of tissue included in the grasp of the clamp was saved for histological examination.

Exp. 18. March 4, 1905. Small fox terrier. Preparation was the same as in preceding experiments. When clamp was put in place and tightened no block resulted. Hook was then withdrawn from the ventricular septum and reinserted in what was thought to be a better position. Now tightening gave complete block. The hook was unclamped immediately and I began to make tests, details of which are given in Table XX. The block did not disappear for some time; the first escape is given in the table.

Autopsy.—The hook was found to occupy the same position as in the preceding experiment. Both coronaries were patent. The tissue included in the clamp was excised for histological examination.

Exp. 19. March 6, 1905. Small dog.

(*a*) I tried to establish block by cooling; a brass tube of 2 mm. bore was bent so that it would fit snugly in the groove between the aorta and the right auricle. After putting it in place I circulated fluid at -3° C. through it for 10 min. without effect upon the sequence of the heart-beat.

(*b*) Clamp was then adjusted on heart. While passing the hook there was a transitory 2 : 1 rhythm. Later (before preparations for making tracing had been completed) 2: 1 rhythm occurred. Now during very weak stimulation of the vagus the rhythm changed to 1: 1, returning to 2 : 1 upon cessation of stimulation. This was repeated several times. Complete block then gradually developed and persisted for about 3 hrs., although the clamp had not been tightened. After recovery from block, tightening of the clamp caused the block to return with great precision. This phenomenon, with other details, is recorded in Table XXI. At the close of the experiment while the block was complete the right coronary artery was plugged by Porter's method. This was associated with no perceptible change in the rate of the ventricles, although the artery was closed for more than 2 min. The heart continued to beat for some minutes after excision.

Autopsy.—The sound is in the right coronary artery. The clamp occupies practically the same position as in preceding experiment. The tissue included in the clamp was excised for histological examination.

Exp. 20. March 7, 1905. Small dog. While passing the hook complete block came on and persisted throughout the course of the experiment. At the close of the experiment the left coronary artery was plugged by Porter's method. Although no record was made of this test, it was perfectly obvious that the rate of the ventricular beat was not materially affected after 4 min. Then the artificial respiration was stopped with the coronary sound still in place. After 3 min. the heart was still beating, and it continued to beat for some time after the heart had been excised, even after opening the right and left ventricles. The details of the experiment are given in Table XXII.

TABLE XX.

Procedures and Remarks.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	$\frac{A}{V}$	Max. Pres.	Min. Pres.
Tracing begun after escape from complete block.										
Stimulation of accel.	Before.	40	66	182	40	66	182	1	96	92
	During.	41	68	181	41	68	181	1	94	90
Same, coil at 11.	Before.	31	51.8	180	31	51.8	180	1	94	90
	During.	26	43.2	181	26	43.2	181	1	93	89
Same, coil at 9.	Before.	27	45.3	179	27	45.3	179	1	93	90
	During.	30	50.1	180	30	50.1	180	1	93	90
Same, coil at 9.	Before.	26	44	177	26	44	177	1	93	90
	During.	28	46.8	179	28	46.8	179	1	93	90
Block developing spontaneously, probably from tension on clamp; first rhythm, 2 : 1, then alternately 1:1 and 2:1, and finally 2:1										
Stim. of accel., coil at 9.	Before.	13	43.6	89.5	—	—	—	2 (?)	90	80
(Auricles not writing.)	During.	13	44	89.7	—	—	—	2 (?)	90	80
Vacillation between 3:1 and complete block.										
Stim. of vagus, coil at 13.	Before.	8	43	55.8	25.3	43	177	3.2	79	58
	During.	8	42.8	56.1	20	42.8	140	2.5	75	56
	After.	7	37.8	55.6	21.8	37.8	173	3.1	78	58.5
Stim. of vagus, coil at 9.	Before.	9	48	56.2	28.5	48	178	3.2	79	58
	During.	9	49	54.9	0	—	—	—	64	51
Stim. of accel., coil at 9.	Before.	13	69	56.5	38	69	165	2.9	74	52
	During.	13	69	56.5	39	69	170	3	76	55
	After.	10	52.6	57	30.3	52.6	173	3	76	56
Stim. of vagus, coil at 9.	Before.	9	48	56.2	26.3	48	164	2.9	74	52
	During.	9	49	55.1	0	—	—	—	63	50
	After.	8	43	55.8	23	43	161	2.9	73	51
Stim of accel., coil at 7.	Before.	8	42	57.1	23.2	42	166	2.9	74	53
	During.	14	74.6	56.3	40.5	74.6	163	2.9	76	54
	After.	11	58	56.9	33.5	58	173	3	75	53
Effect of asphyxia.	Before.	10	55.8	53.8	29.7	55.8	160	2.97	69	51
(Art. resp stopped.)	1st 10 Vs.	10	55.8	53.8	29.3	55.8	157	2.93	69	51
	Before rise of pres.	10	56.8	52.8	29	56.8	153	2.9	67	50
	Pres. rising.	?	—	—	30	61	148	—	max. 107	97
(Art. resp. resumed.)	Pres. falling.	?	—	—	31	58.4	159	—		
	Later.				31	58	160			
After this V becomes irregular: groups of 2 to 3-2:1 alternating with 3:1, or complete block. Pulse and pressure too irregular for measurement.										
Effect of asphyxia.	Before.	10	57.6	52.1	33.3	57.6	173	3.3	77	66
	Art. resp. stopped.	10	58	51.7	33.5	58	173	3.35	80	70
	Pres. rising.	10	57.6	52.1	32.5	57.6	169	3.25	84	74
	Pres. constant.	10	57.8	51.9	31	57.8	161	3.1	83	73
Art. resp. resumed.	Pres. falling.	10	58.2	51	31.7	58.2	162	3.2	77	68
Stim. of vagus, coil at 9.	Before.	12	42	85.7	24	42	171.4	2	89	84
(Occasional As'.)	During.	4	34.2	35.1	—	—	—	—	—	41
	After recovery of auricles.*	29	56.2	155	29	56.2	155	1	91.5	90
Stim. of accel., coil at 9.	Before.	25	44	170	25	44	170	1	73.5	72
	During.	26	50	156	26	50	156	1	76.5	75

* At cessation of stimulation the auricle gave two feeble beats followed by a very strong one, after which it went into a condition resembling fibrillary contractions for about 100/5 sec. When it resumed its normal condition its contractions were at first powerful, but they rather rapidly diminished in height (for about 25/5) and then slowly increased to normal size. At cessation of stimulation the ventricles began to beat with a rate that was faster than before stimulation, and within a few beats the pressure was at its maximum (98–96). When the auricles went into fibrillary contractions the ventricular rhythm became very irregular. It was slowest just before the auricles recovered as with inhibition. When the auricles began to beat the ventricles followed them with a 1 : 1 rhythm.

Autopsy.—The sound is in the left coronary. Position of the clamp is about as in preceding experiments.

Exp. 21. March 8, 1905. Large dog. Insertion of the hook was not associated with any disturbance in sequence. On clamping to determine if the clamp was in the right place, complete block came on at once. At first the ventricular rate was very slow indeed, but it gradually increased until the ventricular rate usual for heart-block obtained. This phenomenon was observed a second time during the course of the experiment. For details, see Table XXIII.

TABLE XXI.

Procedures and Remarks.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	$\frac{A}{V}$	Max. Pres.	Min. Pres.
After adjusting clamp complete block developed.										
Stim. vagus, weak.	Before.	15	65.7	68.5	35.5	65.7	157.5	2.3	81	76
	During.	18	79	68.3	42	79	159.4	2.3	74	68
Stim. vagus, strong.	Before.	16	70.6	68–	37.7	70.6	160.2	2.3	76	70
	During.	11	52	63.5	few,	—	—	—	63	57
Left vagus cut, rt. vagus cooled to −3° C.		17	74.3	68.6	39.8	74.3	160.7	2.3	81	75
Stim. rt. vagus central of cooled area.										
	Before.	10	44.5	67.4	24	44.5	161.8	2.4	84	79
	During.	12	55.4	65	few	—	—	—	66	61
	After.	9	40.7	66.3	21.3	40.7	157	2.4	77	71
Same.	Before.	10	44.8	67	24.6	44.8	164.7	2.5	82	75
	During.	13	59.4	65.6	27	59.4	136.4	2.1	66	60
Same.	Before.	11	50.8	64.9	26.8	50.8	158.3	2.4	81	74
	During.	13	61	63.9	31	61	152.5	2.4	68	62
Stim. rt. vagus after warming.	Before.	10	46.8	64.1	24.6	46.8	157.7	2.5	87	80
	During.	13	63.4	61.5	few	—	—	—	72	64
Stim. accel. several times—negative.										
Cut rt. vagus.	Before.	15	68.8	65.4	35.3	68.8	153.9	2.35	87	79
	After.	16	74	64.8	37.7	74	152.8	2.4	85	76
Block passed off spontaneously: now stim. of vagus gave complete inhib. of A and V.										
Before clamping.		17	55.2	92.4	17	55.2	92.4	1	54	51
Clamping (occasional 2: 1, then:)		12	68.3	52.7	24	68.3	105.4	2	50	43
Now stim. of vagus gives complete inhib. of A and V.*										
Clamping.		9	66.2	40.8	20.4	66.2	92.5	2.3	47	36
Stim. of vagus.		9	69.2	39	0	—	—	—	37	30

Autopsy.—Weight of heart, 94 grm. The hook entered the aorta a little to the right of the line of junction of the right and posterior leaflets of the aortic valve and 8 mm. below the uppermost point of the line of attachment of the leaflets. It entered the muscular septum 2 mm. posterior to the above-mentioned line of junction and on the line of attachment of the lower edge of the posterior leaflet with the muscular septum. It passed obliquely through the septum and appeared in the right ventricle just behind the middle point of

* Fibrillary contractions of auricles occurred for a short time immediately after recovery from inhibition.

the mesial leaflet of the tricuspid valve, about 3 mm. below the auriculo-ventricular junction. The tissue included in the clamp was saved for histological examination.

TABLE XXII.

Procedures and Remarks.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	$\frac{A}{V}$	Max. Pres.	Min. Pres.
Stimulation of vagus.	Before.	8	50.2	47.8	26.4	50.2	157.7	3.3	94	88
	During.	6	39.8	45.2	0	—	—	—	77	72
Stim. of accel.	Before.	15	86.6	51.96	47.8	86.6	165.6	3.2	81	72
	During max. accel.	11	61	54.59	36.5	61	179.5	3.3	79	70
	After.	7	38.4	54.7	22.7	38.4	177.4	3.24	82	73
Stim. of accel.	Before.	11	59.3	55.65	33.3	59.3	168.5	3.03	74	66
	During max. accel.	13	65.4	59.6	41.3	65.4	189.4	3.18	82	74
	After.	7	35.2	59.66	21.6	35.2	184.1	3.09	71	64
Stim. of accel.	Before.	10	51.8	57.9	28.9	51.8	166.8	2.88	73	67
	During max. accel.	12	57.5	62.6	36.9	57.5	192.5	3.07	77	71
	After.	12	56.8	63.4	34.8	56.8	183.8	2.9	68	63
Ligation of both vagi.	Before.	11	53.7	61.4	29	53.7	162	2.64	68	62
	After.	11	53.4	61.8	29.3	53.4	164.6	2.66	60	55
Stim. of accel.	Before.	11	53.3	61 9	31.5	53.3	177.3	2.86	57	52
	During.	10	48.3	62.1	23.5	48.3	146	2.35	64	58
		10	47.8	62.7	29.3	47.8	183.9	2.93	66	60
	After.	14	65.8	63.8	39.8	65.8	181.4	2.84	54	49
At beginning of stim. auricles were slightly inhibited (escape) but V. slightly accelerated and pulse pressure and blood pressure increased.										
Stim. of accel.	Before.	10	49.2	61	26.2	49.2	159.7	2.62	57	51
	During.	17	82.6	61.7	45	82.6	163.4	2.65	61	56
	After.	16	79	60.8	42.6	79	161.8	2.66	54	49

Exp. 22. March 17, 1905. Small dog. While making the preliminary dissection a vessel in the auriculo-ventricular groove was torn; the hæmorrhage that resulted was severe and could not be checked. The hook was, however, inserted. The clamp was closed suddenly with the fingers, but no block resulted. The hook was then withdrawn from the ventricular septum and reinserted. While recording the ventricular contractions alone (it was feared that the animal might die from hæmorrhage if time was taken to arrange the auricular recording apparatus), the clamp was again suddenly closed with the fingers. The ventricle at once came to rest for 22 sec., and then very gradually developed a rapid rate which was independent of that of the auricles. During this time there was no obvious change in the auricular rate. The complete block persisted after unclamping. Then the clamp was again suddenly tightened to see if irritation would cause stoppage of the ventricles. It caused an extra-systole only.

Autopsy.—The hook entered the muscular septum on the line of junction of the right and posterior leaflets of the aortic valve and emerged in the right ventricle 5 mm. below the auriculo-ventricular junction and about 5 mm. posterior to the anterior edge of the mesial leaflet of the tricuspid valve.

TABLE XXIII.

Procedures and Remarks.		No. of Vs.	Duration in $\frac{1}{5}$ Sec.	V. Rate per Min.	No. of As.	Duration in $\frac{1}{5}$ Sec.	A. Rate per Min.	$\frac{A}{V}$	Max. Pres.	Min. Pres.
Stimulation of vagus.		0		inhib.	0		inhib.			
Stim. of accel.	Before.	28	75.2	111.7	28	75.2	111.7	1	119	115
	During.	34	89.2	114.4	34	89.2	114.4	1	129	125
	After.	27	70.1	115.5	27	70.1	115.5	1	113	109
Stim. of accel.	Before.	20	53	111.2	20	53	111.2	1	130	126
	During.	43	101.3	127.3	43	101.3	127.3	1	146	143
	After.	25	61.8	121.4	25	61.8	121.4	1	133	130
Clamped; complete block at once with preliminary slowing of V. Tracing not begun at once. The 1st Vs. recorded=(earlier Vs. much longer) V. rate then gradually increased until as below.		1	27.5	—	11	27.5	120	—	—	18
Stim. of vagus.	Before.	8	53.5	44.9	21	53.5	117.8	2.6	116	98
	During.	12	86	42	0	—	—	—	93	76
	After.	11	73.8	44.7	?	—	—	—	107	92
Stim. of accel.	Before.	13	88.3	44.2	34.7	88.3	117.9	2.7	109	92
	During.	17	115.7	44.1	50.4	115.7	130.7	2.96	126	110
	After.	10	69.4	43.2	32	69.4	138.4	3.2	105	89
Stim. of accel.	Before.	10	71.1	42.2	31.7	71.1	133.7	3.2	114	108
	During.	13	86.6	45	38.7	86.6	134	2.98	121	112
	After.	11	71.5	46.2	32.2	71.5	135.1	2.93	127	115
Stim. of accel.	Before.	9	65	41.5	?	—	—	—	123	113
	During.	13	86.3	45.2	?	—	—	—	116	102
Stim. of accel.	Before.	13	97.2	40.1	43.6	97.2	134.6	3.4—	122	109
	During.	12	82.3	43.7	36.8	82.3	134.2	3.07	120	105
	After.	10	68.2	44	30.6	68.2	134.6	3.06	121	110
Stim. of accel.	Before.	8	58	41.4	25.6	58	132.4	3.2	122	112
	During.	12	80.7	44.6	35.9	80.7	133.2	2.97	125	117
	After.	12	80.2	44.9	36	80.2	134.6	3.00	127	118
Stim. of accel.	Before.	11	79.7	41.4	35.6	79.7	134	3.24	113	90
	During.	15	101.2	44.5	45	101.2	133.6	3.00	122	103
	After.	10	68.2	44.4	29.8	68.2	131	2.95	117	98
Stim. of vagus.	Before.	9	65.4	41.3	29	65.4	133	3.22	112	91
	During.	10	76.7	39.1	0	—	—	—	98	78
Bleeding rapidly from femoral artery.										
	Before.	11	71	46.5	30.5	71	128.9	2.77	118	104
	At beginning.	10	68	44.1	29	68	127.9	2.9	88	74
	Near middle.	11	69	47.8	28.3	69	123.1	2.6	44	37
	Near end.	9	57.2	47.2	20	57.2	104.9	2.22	18	16

Exp. 23. March 24, 1905. Small dog. No tracing was made. The hook was inserted without difficulty and without disturbing the normal sequence. The clamp was then carefully tightened with the fingers (the nut was not used). A short period of a 2 : 1 rhythm was followed by stoppage of the ventricles which must have lasted fully 20 sec. The ventricular rate then gradually increased, and when it became constant the block was complete and the rate of the ventricles was about usual for complete heart-block. The auricular rate was not obviously disturbed. Upon unclamping, the block disappeared promptly through a short period of a 2 : 1 rhythm. The clamp was again tightened as before with exactly the same result. The clamp was then loosened,

and while the block was still complete the clamp was suddenly and sharply tightened. The ventricles responded with several extra-systoles; nothing resembling stoppage occurred.

Autopsy.—The hook entered the left ventricle at about the middle point of the posterior half of the right aortic leaflet; entered the muscular septum about 5 mm. below and to the left of this point and emerged into the right ventricle at a point slightly anterior to the middle point of the mesial tricuspid leaflet. The tissue included in the clamp was removed for histological examination.

Exp. 24. March 25, 1905. *Effect of electrical stimulation of the tissue included in the clamp.* For this and a few of the subsequent experiments a "clamp electrode" was used. The brass block was replaced by a block of hard rubber. To its lower edge a small plate of platinum was attached. This was united with a fine binding post on the upper edge of the block. All of the fish-hook, excepting 2 or 3 mm. near the point, was coated with a heavy layer of shellac. The upper end of the brass bar into which the hook is soldered was converted into a binding post. These two binding posts were connected with the terminals of a secondary coil. When tested on the tongue it was found that induction shocks passed through only that part of the tongue which was included between the platinum plate and the exposed part of the hook.

The hook was inserted. Upon tightening the clamp very gently with the fingers I obtained a 2 : 1 rhythm, and then released immediately. I stimulated through the clamp with the coil at twenty-seven. The auricles went into fibrillary, or at least very rapid, contractions, and did not recover for 45 min. The ventricles continued to beat, but quite irregularly. When the heart became normal a record was made of the effect of stimulating through the clamp. (Results were neither constant nor clear, and hence are not given here.) The clamp was then tightened until the rhythm became 2 : 1, and later until block became complete. The phenomenon of stoppage of the ventricles was not obtained.

Autopsy.—Hook entered through posterior part of right aortic leaflet and passed into the septum about 7 mm. below the highest point of attachment of the valves and on their line of junction. It ran through the septum quite obliquely posteriorly and emerged in the right ventricle about 6 mm. below the auriculo-ventricular junction and a little anterior to the middle point of the mesial tricuspid leaflet.

Exp. 25. March 28, 1905. Very large dog; had previously been used in another experiment in which atropine had been injected intravenously. Strongest stimulation of the vagus was without effect on the heart-rate. No tracings were made. Clamp electrode was placed on the heart and tightened with the fingers, but no block resulted. Clamp was reinserted in the septum and again tightened with fingers; a few beats with 2: 1 rhythm and then complete block resulted. The ventricular rate at first was rather slow and gradually increased to a uniform rate of something between a 2: 1 and 3: 1 rhythm. Although the ventricular rate was at first slow, it was not exactly characteristic of the phenomenon of stoppage. The clamp was then loosened, and while the rhythm was passing through a 2: 1 to normal rhythm, the tissue included in the clamp was stimulated with tetani. With weak currents the rate of both auricles and ventricles was apparently increased. However, this was not very marked

even with strong currents—up to twelve. The clamp was again tightened until complete block came on. It was then loosened somewhat, but the block remained complete. Stimulation with increasing strengths of current was again tried. All strengths, but especially the strong, markedly increased the rate of the ventricles. The ventricles fluttered, but the contractions were not fibrillary. The increased rate of the ventricles ceased synchronously with the cessation of stimulation, at least, in most instances. This continued until the coil was at 11.5, when the ventricles went into fibrillary contractions.

Autopsy.—The hook entered the aorta near the middle point of the posterior half of the right aortic leaflet. It entered the septum on the line of junction of the right and posterior leaflets about 4 mm. below the uppermost point of attachment. It emerged in the right ventricle about 3 mm. below the auriculo-ventricular junction and about 3 mm. posterior to the anterior edge of the mesial tricuspid leaflet. The tissue included in the clamp was excised for histological examination.

Exp. 26. March 30, 1905. Medium-sized dog. The clamp electrode was adjusted on heart and tightened with fingers; no block resulted. Hook was reinserted in septum and clamp again tightened with fingers; complete block came on at once preceded by the phenonemon of stoppage. When hook was unclamped, normal sequence returned. I now began to make tracings. At various times the tissue in the clamp was stimulated with tetani of increasing strength. The results were not constant and have not been included in the analysis given below.

Analysis of Tracings of Exp. 26.—Clamp tightened: complete block came on at once, preceded by marked preliminary slowing of the ventricles. 1st ventricular cycle lasted 14.1 sec., and in it blood-pressure fell to 21 mm. Hg. During this ventricular cycle the auricular rate was 145.7 per min. (The auricular rate had been 154.3 before clamping.) Ventricular rate then gradually increased and when it became constant it was 29.7.

Hook unclamped: 2 : 1 rhythm came on and persisted.

Atropine (4 mgr.) administered intravenously.

Stimulation of vagus: no slowing of auricle or ventricle.

Hook clamped (rhythm still 2 : 1): 1stventricular systole somewhat longer than others, but I cannot be certain that complete block was obtained because ventricular rhythm was not regular.

Hook unclamped: 2 : 1 rhythm.

Stimulation of vagus: no slowing of auricle or ventricle.

Hook clamped: 3 : 1 rhythm (apparently—auricular tracing not distinct).

Hook unclamped: auricles too weak to record.

Autopsy.—The hook entered the aorta exactly as in the preceding experiment. It entered the septum on the line of junction of the two leaflets and 7 mm. below their uppermost line of attachment. It emerged in the right ventricle about 5 mm. below the auriculo-ventricular junction and about 5 or 6 mm. posterior to the anterior edge of the mesial tricuspid leaflet.

Exp. 27. March 31, 1905. Large dog. Hook inserted and clamp tightened with fingers—no block. Hook withdrawn from septum and reinserted—complete block without stoppage. Tracings were then made with the Hürthle manometer. In this experiment, upon tightening the clamp complete block

never came on at once, indeed there was an unusually wide margin between the normal sequence and the various stages of block.

Autopsy.—The hook entered the aorta rather low and somewhat anterior to the line of junction of the right and posterior aortic leaflets. It entered the septum 2 or 3 mm. posterior to this line and about 8 mm. below the uppermost line of attachment of the valves. It entered the right ventricle 4 mm. below the auriculo-ventricular junction and about 6 mm. posterior to the anterior edge of the mesial tricuspid leaflet.

Results.—The clamp electrode was used in this experiment and the tissue included in it was stimulated with various strengths of current. The results obtained were not consistent; they have not received a satisfactory interpretation, and therefore will not be presented now.

While unclamping I obtained one ventricular silence in 17, 20, 24, and 23 auricular cycles.

While clamping (with rapidly revolving drum) 1: 1 rhythm passed into alternation of 2–1: 1 with 1–2: 1. Then a 3: 1 rhythm developed and continued through a long period of clamping. It was with great difficulty that complete block was obtained.

A record was then made upon the rapidly revolving drum of the recovery from this block. The following were obtained: 3: 1 and 2: 1 rhythms; one ventricular silence in ten, nine, eight, seven, six, five, four, and three auricular beats.

Careful measurement of these tracings shows that the ventricular systole which follows each silence comes sooner after its corresponding auricular systole than the other ventricular systoles. In the succeeding cycles the ventricular systoles follow their auricular systoles later and later until another ventricular systole is dropped. The lengthening of the intersystolic interval is most rapid in the cycles which immediately precede the ventricular silences.

Exp. 28. April 4, 1905. Small dog. Clamp was adjusted on heart. When it was tightened with the fingers the sequence of the heart-beat did not change. The hook was withdrawn from the septum and reinserted. Now when the clamp was tightened with the fingers complete block resulted without a tendency toward stoppage. Stimulation of the vagus inhibited the auricles, but not the ventricles. After unclamping the sequence slowly returned to normal. The hook was again withdrawn from the septum and reinserted farther anteriorly. Now upon tightening the clamp with the fingers, complete block came on with some, although not marked, preliminary slowing of the ventricles. When the hook was unclamped, normal sequence returned. Atropine, 2 mgr., was injected directly into the heart. Stimulation of the vagus caused no inhibition. The clamp was tightened with the fingers and complete block without any obvious tendency toward stoppage resulted. When the hook was unclamped, sequence became normal. Clamp was tightened with results same as before.

Autopsy.—The hook entered the aorta between the orifice of the right coronary artery and the posterior edge of the right leaflet. It entered the muscular septum about 5 mm. below the highest point of union of the right and posterior aortic leaflets. The point was found in the right ventricle about 5 mm. below the auriculo-ventricular junction and a few millimeters posterior to the anterior edge of the mesial tricuspid leaflet.

The first position of the hook in the septum was marked by thrombi adhering to the torn endocardium. These were somewhat posterior to the first position and about 1 cm. below the uppermost point of attachment of the aortic leaflets.

Exp. 29. April 5, 1905. Small dog. The clamp was adjusted on the heart and tightened with the fingers: complete block was obtained with intermediate stages and without preliminary stoppage. The clamping was repeated several times with similar results.

The hook was withdrawn and reinserted into the septum. Upon tightening the clamp with the fingers I obtained complete block with short intermediate stages and with a short preliminary stoppage. The complete block did not disappear for about an hour after unclamping.

Autopsy.—The hook slipped out of the septum while excising the heart. From the position of the holes in the septum it was judged that the hook had passed through the septum about in the line of junction of the right and posterior aortic leaflets and had entered the right ventricle from 2 to 3 mm. below the auriculo-ventricular junction.

Exp. 30. April 12, 1905. Large dog. The clamp was put in place and tightened, but no block resulted. The hook was then reinserted in the septum. Now when it was tightened with the fingers there were several extra-systoles, followed by complete stoppage of the ventricles for twenty-nine auricular beats. The ventricular rate gradually increased until there was about one ventricular systole for two auricular systoles and although the clamp was immediately opened the block remained complete for about a half-hour. During this time stimulation of the vagus inhibited the auricles, but not the ventricles. Then an inconstant 2 : 1 rhythm developed. Now stimulation of the vagus inhibited the auricles as before, the ventricles for a short time (about 4 sec.). While the heart was recovering from inhibition it frequently passed through a 1 : 1 rhythm.

The 2 : 1 rhythm was maintained for about 30 min. and then gradually returned to complete block, from which the heart did not recover. After the lapse of another hour the clamp was abruptly tightened with the fingers. Extra contractions of the ventricles were obtained, but no stoppage.

Autopsy.—While excising the heart the hook slipped out of the septum. The position it had occupied was marked by hæmorrhage into the heart muscle. As viewed from the right side the hæmorrhage was at about the middle of the posterior edge of the membranous septum and extended about 3 mm. into the muscular tissue. No hole could be found in the septum corresponding with the first position of the clamp.

Exp. 31. April 15, 1905. Small dog. Two attempts to produce block failed. The hook was then entirely withdrawn from the heart and reinserted anew. Now upon tightening the clamp with the fingers I obtained partial block for a few beats and then complete block without marked preliminary slowing of the ventricles. When the hook was released, at once normal sequence returned. I tried to determine the weakest stimulation of the vagus which would inhibit the ventricles in the different rhythms, but these could not be maintained long enough to allow of satisfactory determinations.

I then used strong stimulation of the vagus and determined the duration of inhibition in the different rhythms.

Upon unclamping complete block was obtained preceded by a few cycles of 2: 1 rhythm. Then I unclamped hook.

Position of coil.	Rhythm.	Duration of inhibition of ventricles in seconds.
11	2: 1	5.
11	1: 1	9.2
11	1: 1	19.4
11	1: 1	20.4

I tightened clamp carefully with screw: complete block without intermediate stages and with a rather long preliminary stoppage of ventricles followed. I unclamped at once: recovery was very rapid. The following were obtained within a few seconds after recovery from complete block:

Position of coil.	Rhythm.	Duration of inhibition.
11	1: 1	3.
11	1: 1	4.2
11	1: 1	4.4
11	1: 1	4.4

Upon clamping I got complete block at once with preliminary stoppage of the ventricles, the longest ventricular cycle lasting twenty-one auricular systoles (auricular rate = 20 in 16 sec.). Upon unclamping normal sequence returned very quickly: immediate stimulation of vagus with coil at four inhibited the ventricles 3.8 sec. Then:

Position of coil.	Rhythm.	Duration of inhibition.
8	1: 1	4.8
8	1: 1	4.2

Upon clamping I got complete block at once with the following time relations:

No. of ventricular systole:	1st.	2d.	3d.
Duration in auricular systoles.	4	23	longer, and then ventricular rate gradually increased.

Heart unclamped, during recovery:

Position of coil.	Rhythm.	Duration of inhibition.
8	2: 1	4.2

Upon clamping I got complete block with preliminary stoppage of ventricles as follows:

No. of ventricular systole:	1st.	2d.	3d.	4th.
Duration in auricular systoles. .	5	22	9	4

I unclamped immediately and before complete block had passed away clamped sharply and suddenly: nothing obvious happened. Upon unclamping the heart did not recover from complete block.

Every time the clamp was carefully tightened, particularly toward the close of the experiment, the following events were noted: After screwing down to a certain point it could be seen that the intersystolic interval increased in

length. At this time the contractions of the ventricles became peculiar: although the rhythm and rate of contractions remained unchanged, the ventricular muscle showed a curious twisting or writhing motion such as is sometimes seen before the ventricles begin to fibrillate. When this stage was reached the nut was driven down about one half a turn at a time with long pauses between. It was always immediately after the completion of one of these half-turns that the complete block suddenly came on.

Autopsy.—The hook had entered the aorta near the posterior edge of the right leaflet and entered the septum below the line of junction of the right and posterior leaflets, and 8 mm. below their uppermost point of attachment. The point emerged into the right ventricle on a line perpendicular to the middle point of the posterior edge of the membranous septum and about 3 mm. from this edge.

No marks could be seen in the septum of the earlier positions of the clamp and it is therefore probable that it had not entered the septum at all.

EXPLANATION OF PLATES I–IV.

Fig. 20.—Showing different stages of recovery from a 2 : 1 rhythm. Upper tracing = auricular beats (down strokes). Lower tracing = pressure in the central end of the carotid artery with the Hürthle manometer. Time = $\frac{1}{5}$ sec.

A = 1 ventricular silence in 2 auricular cycles.
B = 1 " " 3 " " .
C = 1 " " 4 " " .
D = 1 " " 5 " " .
E = 1 " " 6 and 7 auricular cycles.
F = 1 " " 8, 9, and 10 auricular cycles.
G = Occasional ventricular silences and the return to normal.

Fig. 21.—Showing the usual effect of clamping and unclamping. A = normal. B = 2 : 1 rhythm. C = 3 : 1 rhythm. D = complete block. E = 3 : 1 rhythm. F = 2 : 1 rhythm. G = normal.

Fig. 22.—Cardiogram of complete heart-block in the dog.

Fig. 23.—Showing the effect of stimulation of the vagus in various stages of heart-block. Upper tracing = auricular contractions. Lower = pressure in the central end of the carotid artery with a mercury manometer. Time on base line = $\frac{1}{5}$ sec. A = during normal sequence. B = during 2 : 1 rhythm. C = during complete block.

Fig. 24.—Showing the effect of stimulation of the vagus during normal sequence (A and C) and during complete block (B): otherwise same as Fig. 23.

Fig. 25.—Showing the effect on the pulse-pressure (ventricular output) of an escape auricular beat during vagus inhibition. The heart-block is complete. Otherwise same as Fig. 23.

Fig. 26.—Showing one of the effects of gradually clamping the region of the auriculo-ventricular bundle—ventricular stoppage associated with the sudden development of complete heart-block. Otherwise same as Fig. 23.

Fig. 27.—Jugular sphygmogram (upper) and cardiogram (lower) showing a regularly recurring ventricular extra-systole.

C

F

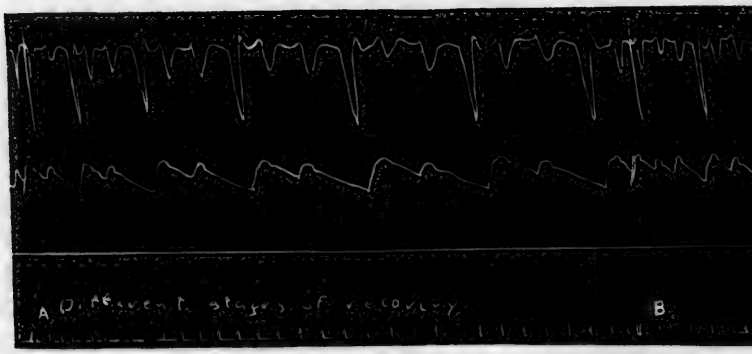


FIG. 20

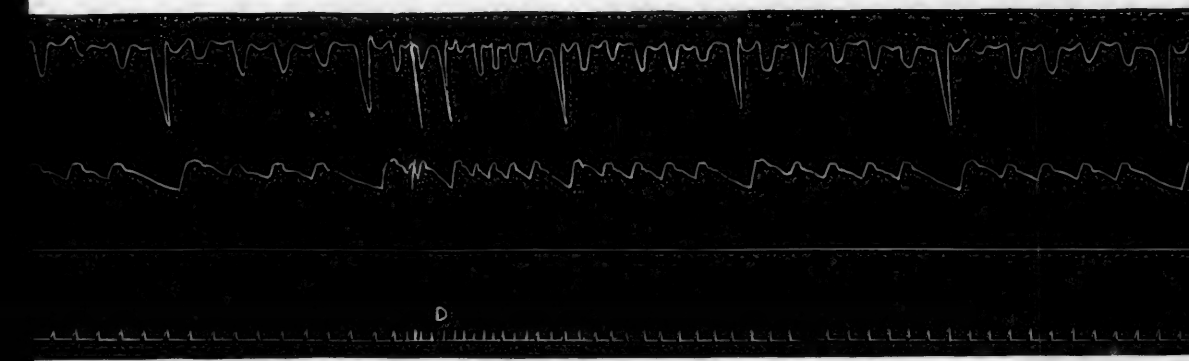


FIG. 20 (CONT

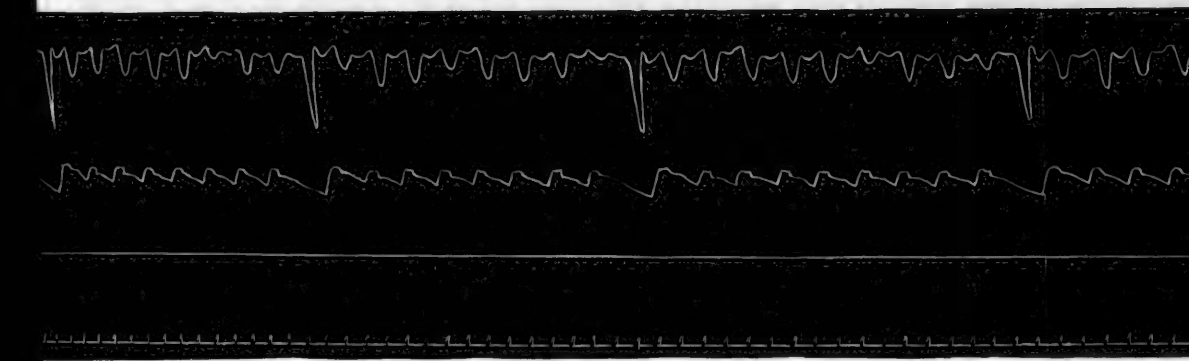
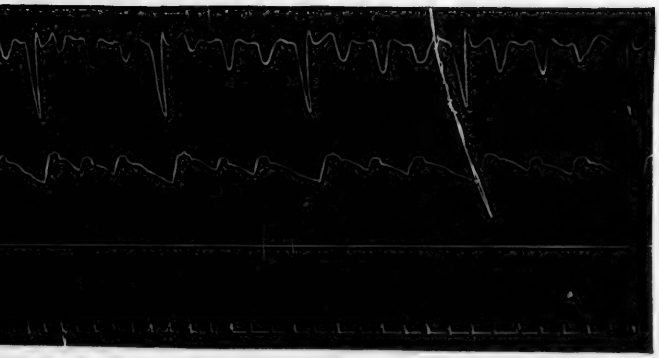
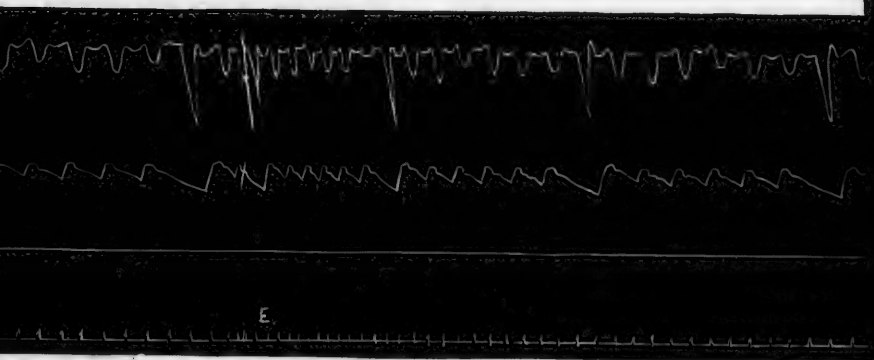


FIG. 20 (CONT





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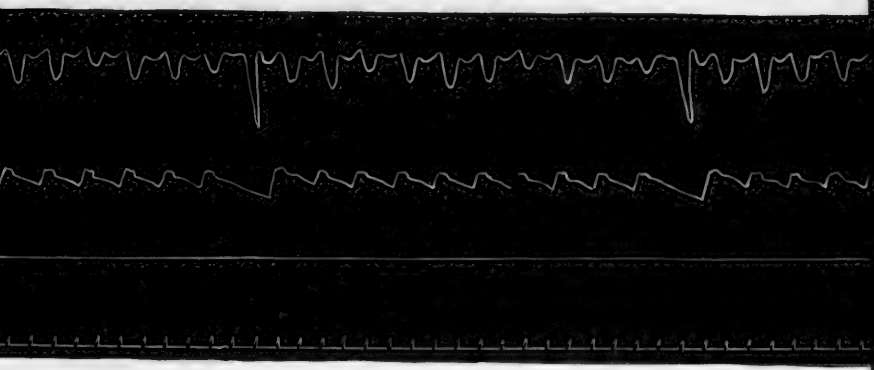

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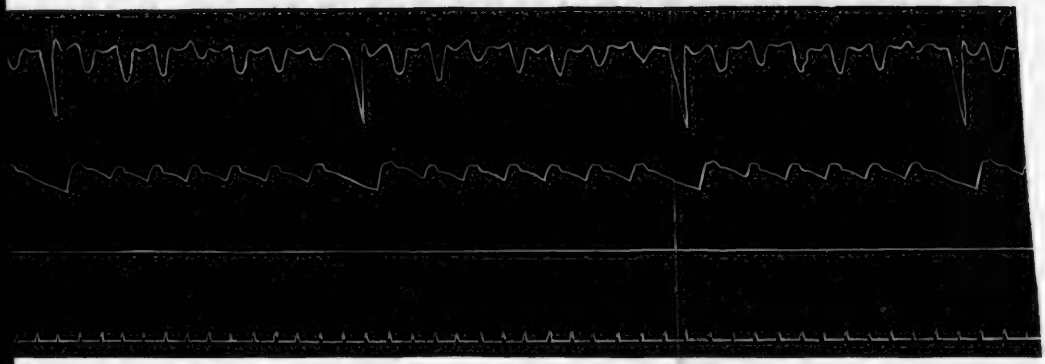
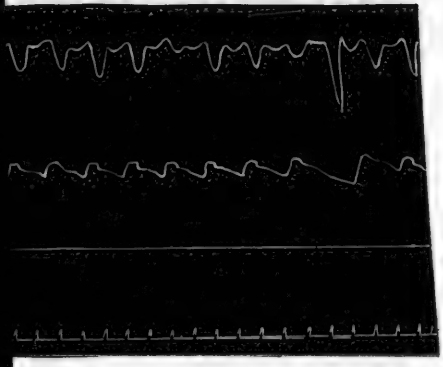
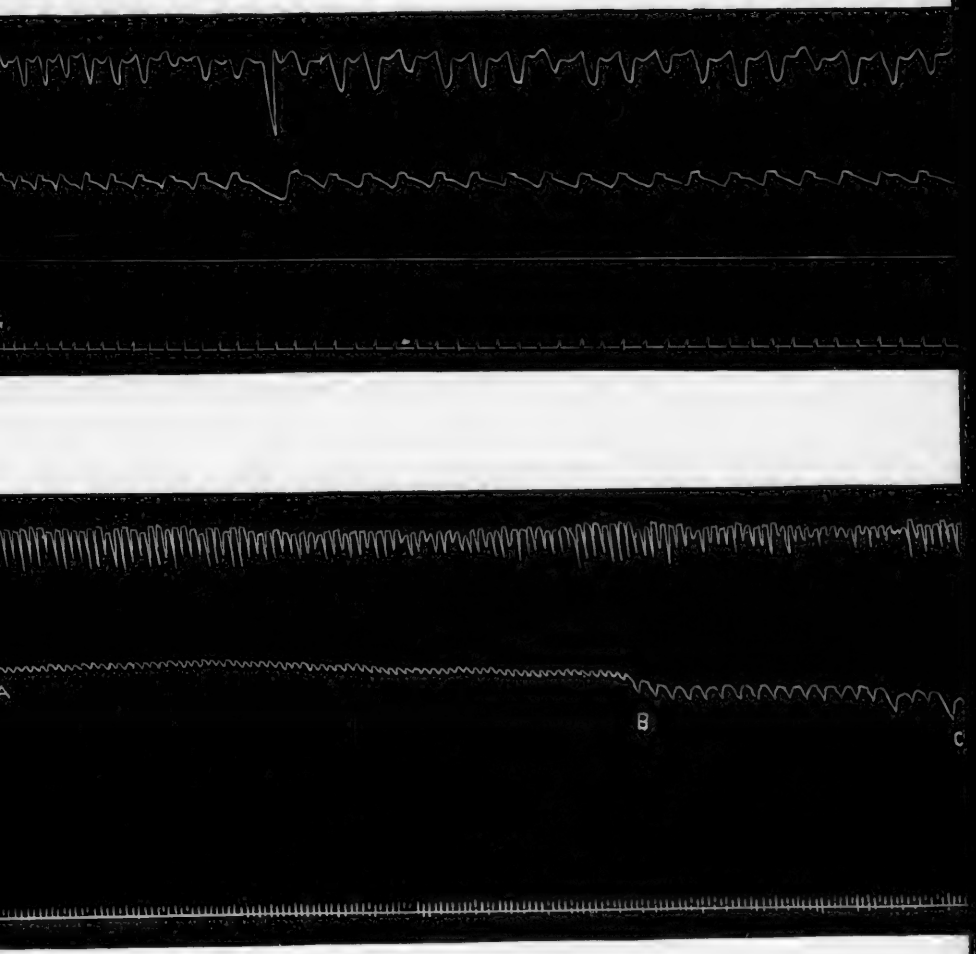
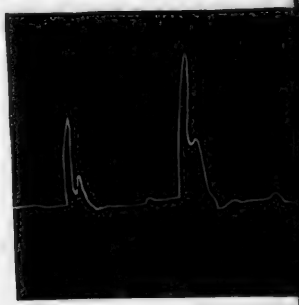





A
B
C



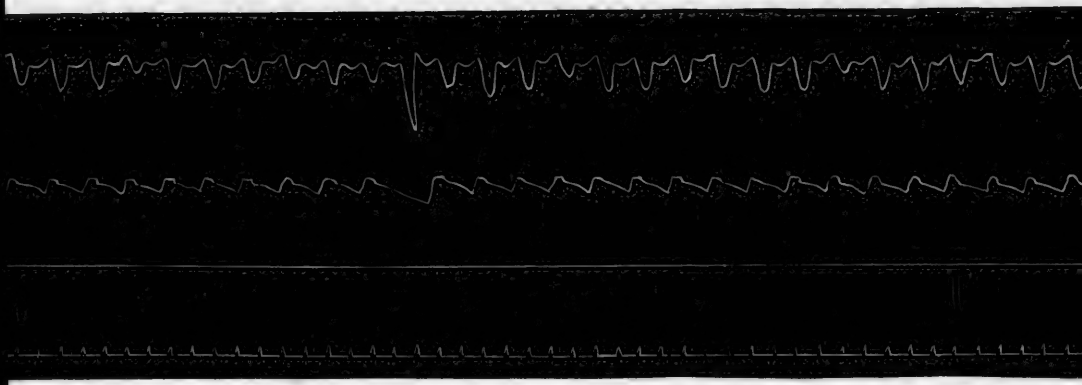


FIG. 20 (CONTINUED)

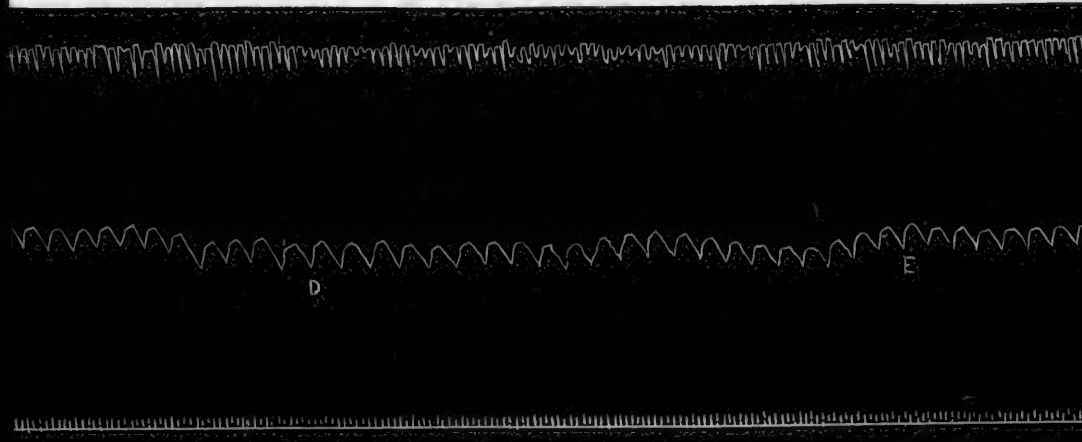


FIG. 21

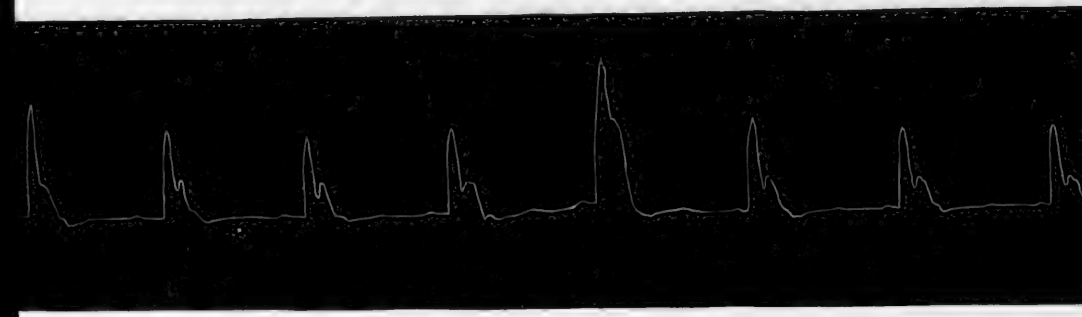
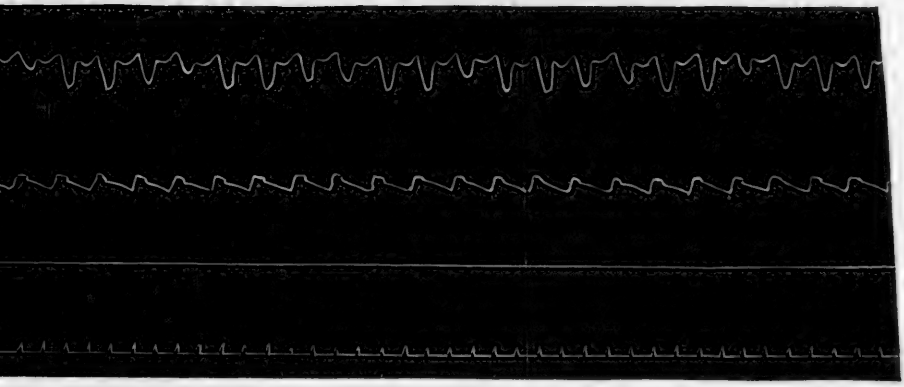
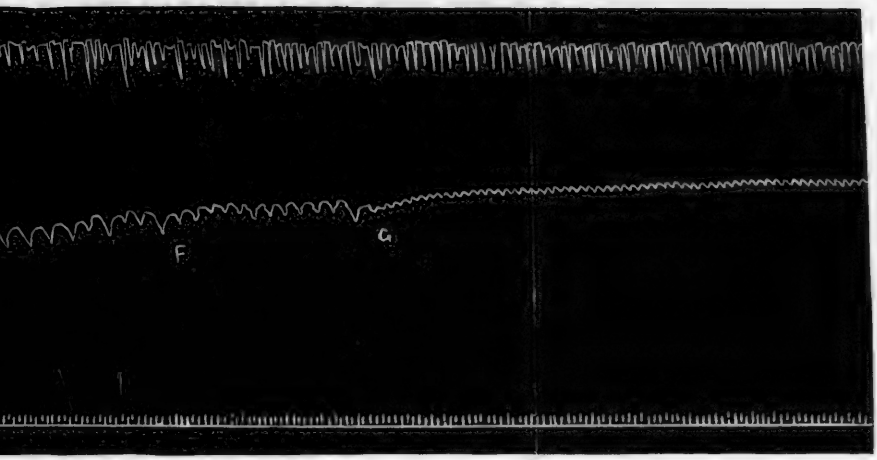
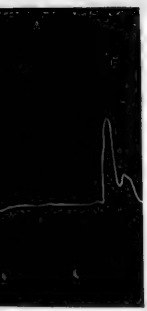


FIG. 22

PLATE II.







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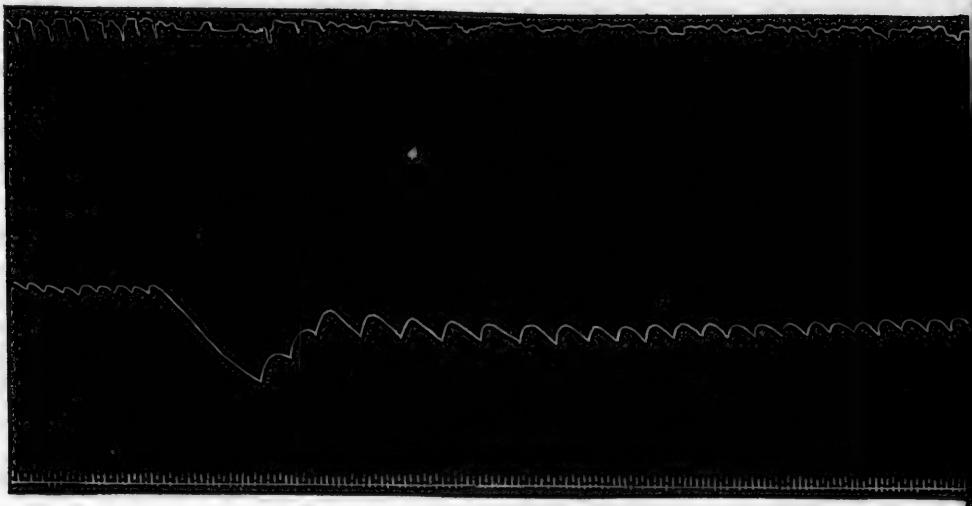
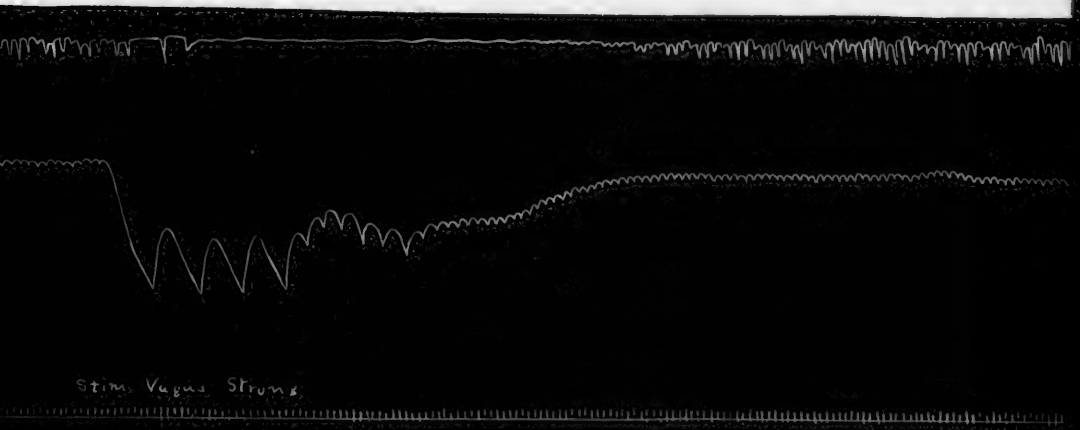


FIG. 23, A



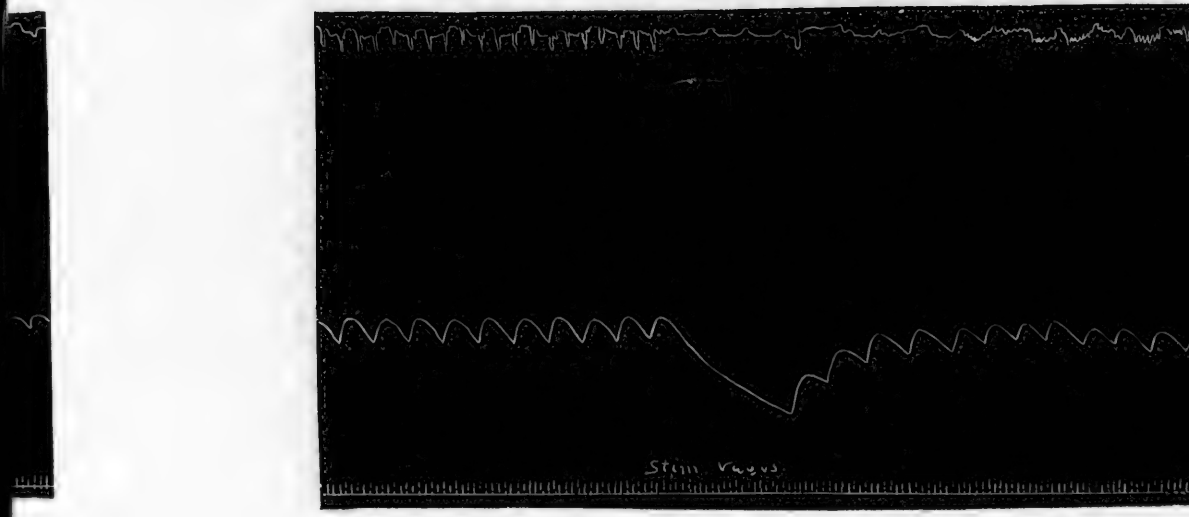


FIG. 23, B

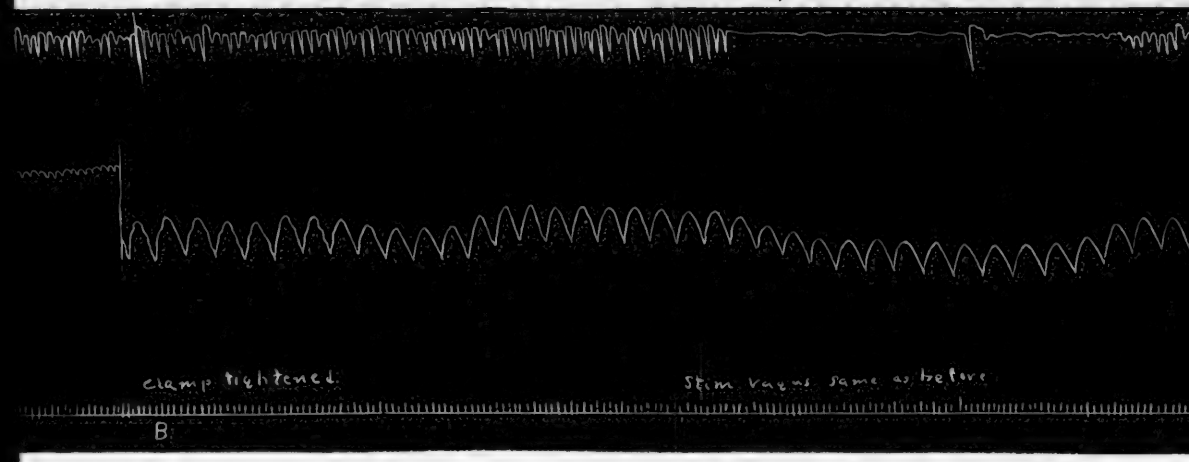


FIG. 24

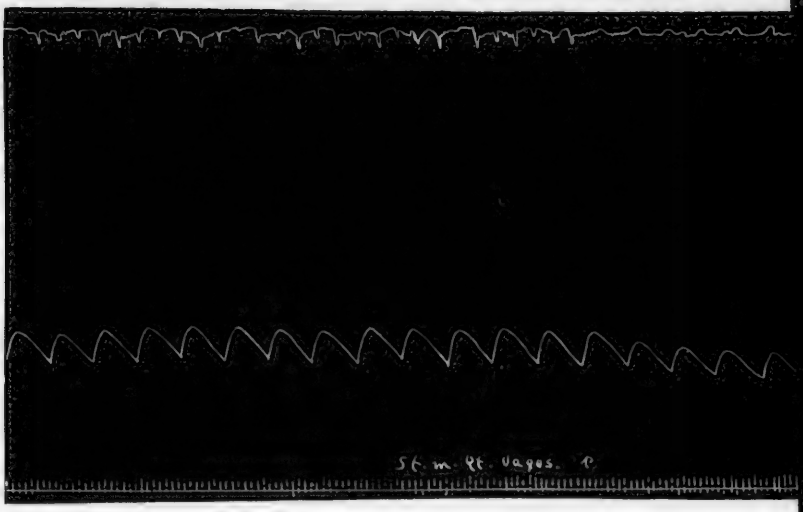
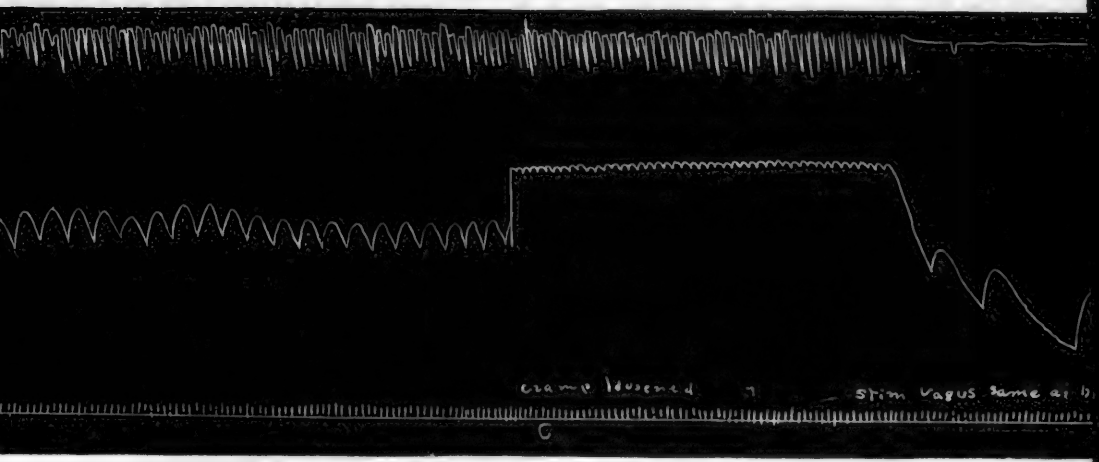


FIG. 23, C



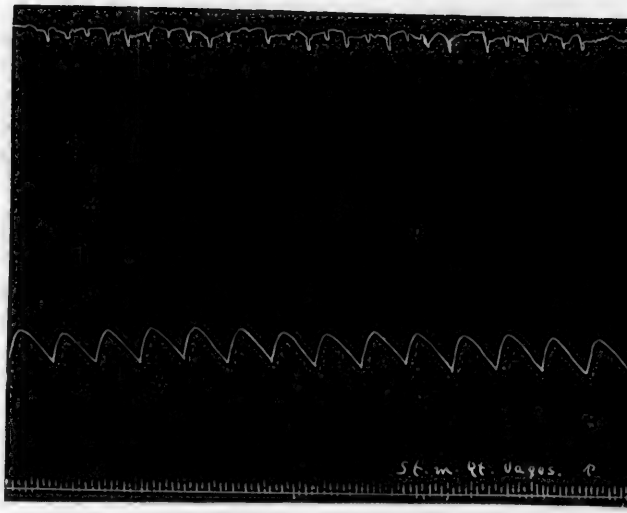


FIG. 23, C

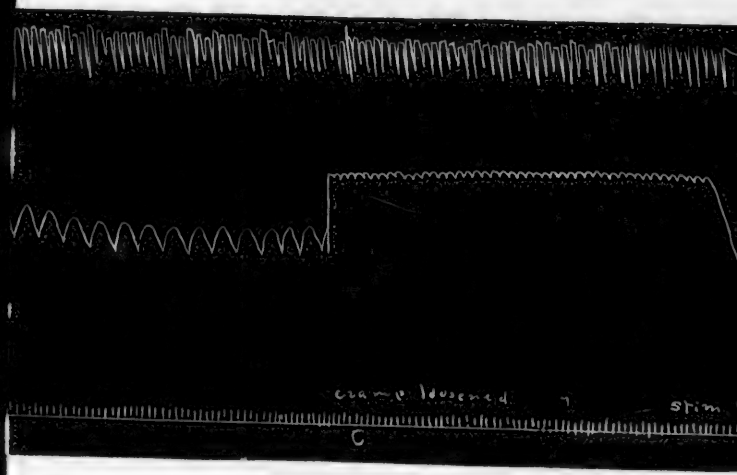
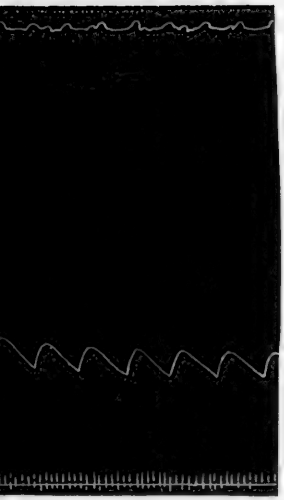
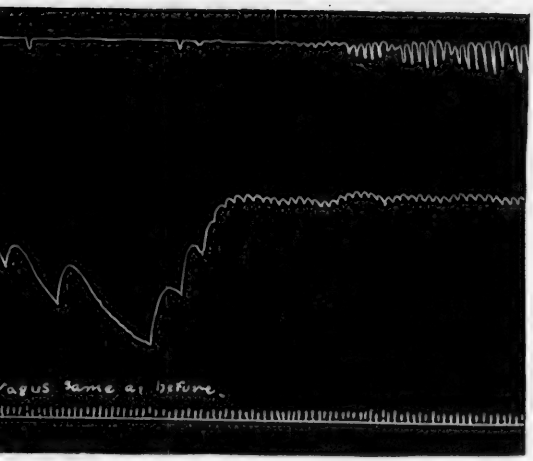


PLATE III.





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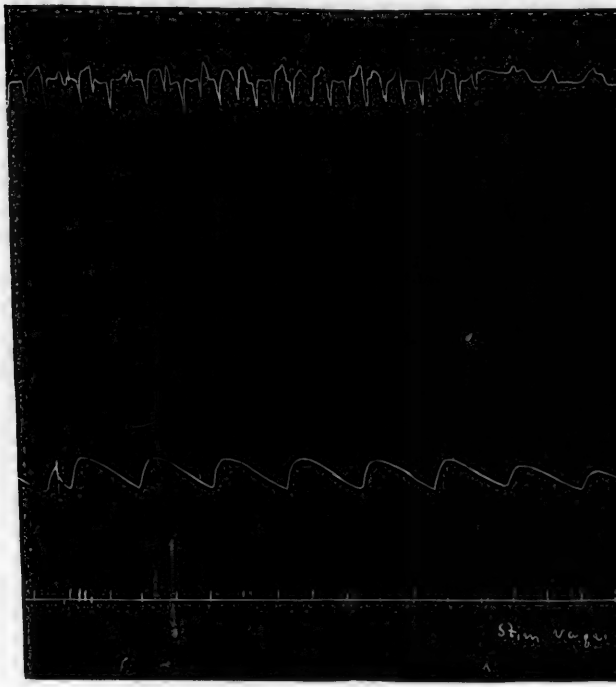
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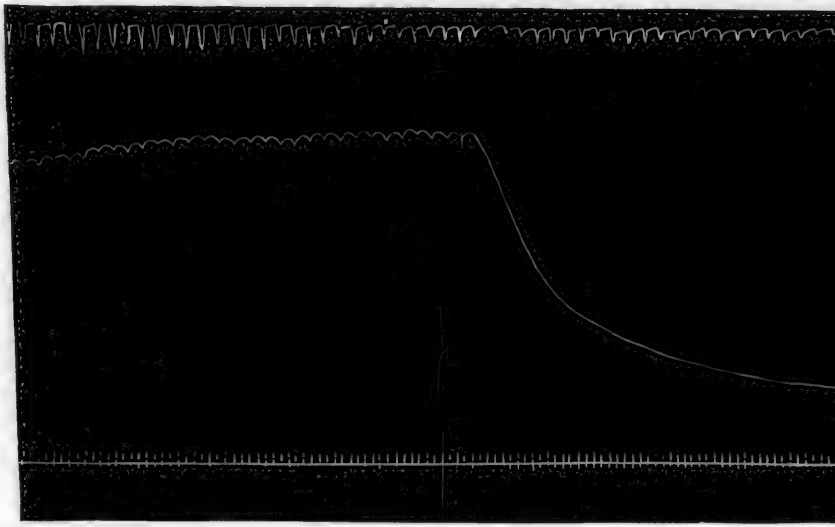


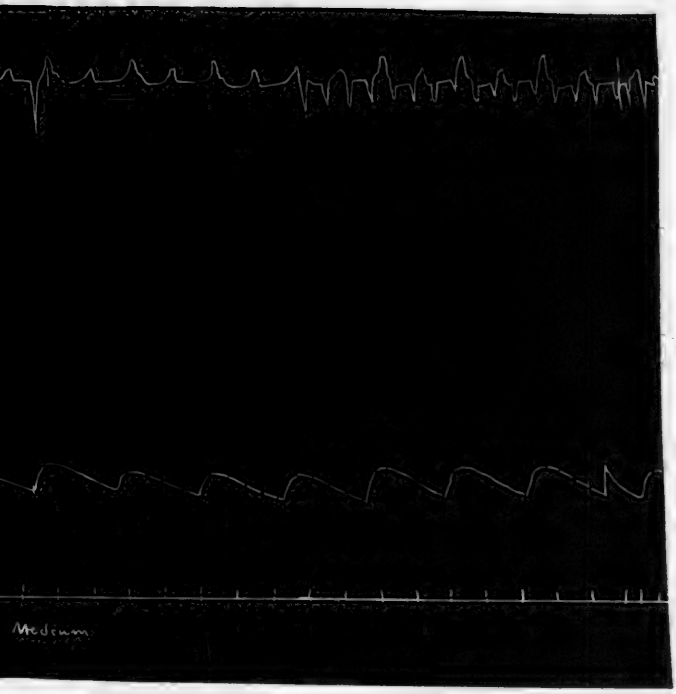
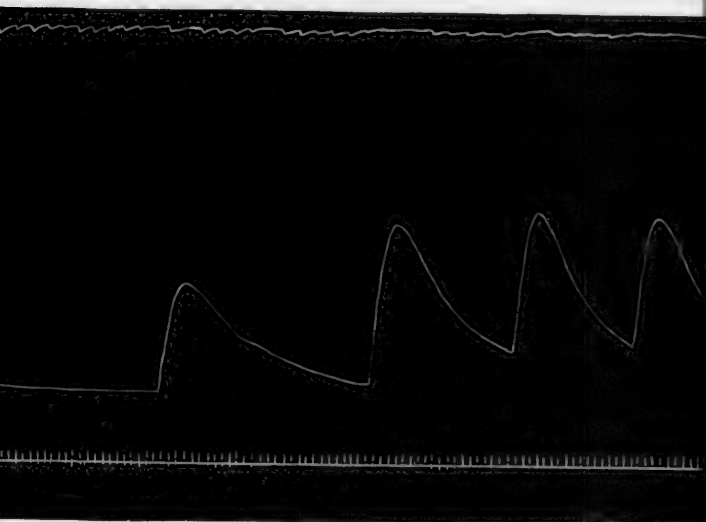


FIG. 25



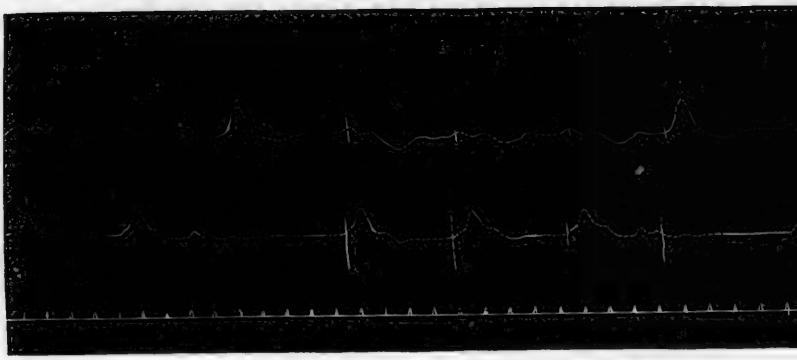


FIG. 27

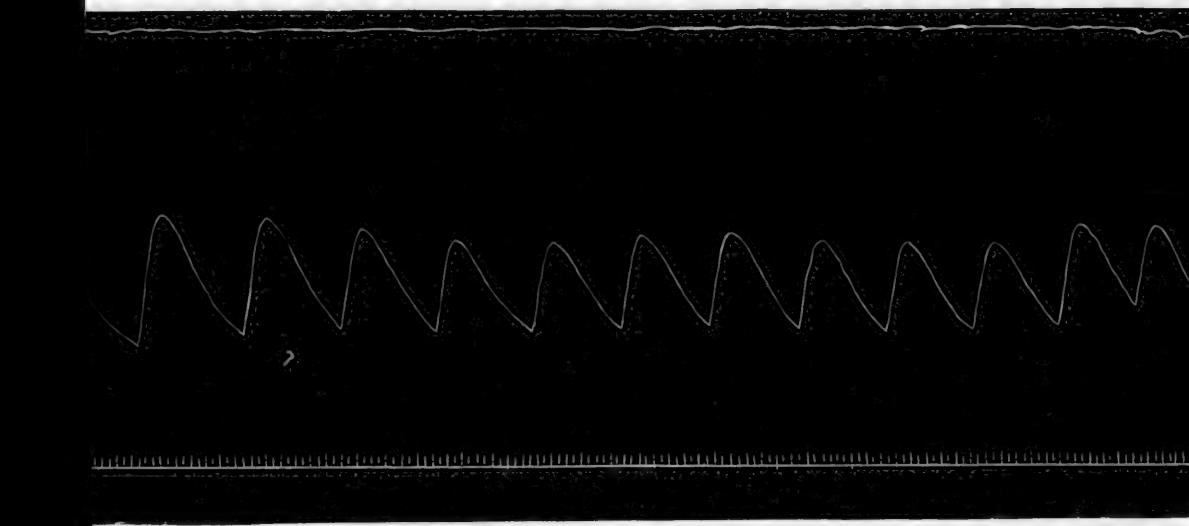
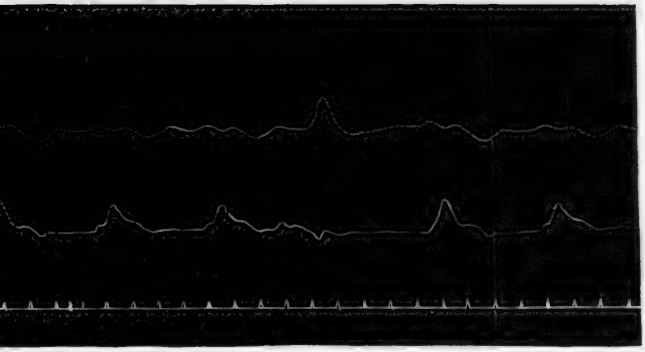
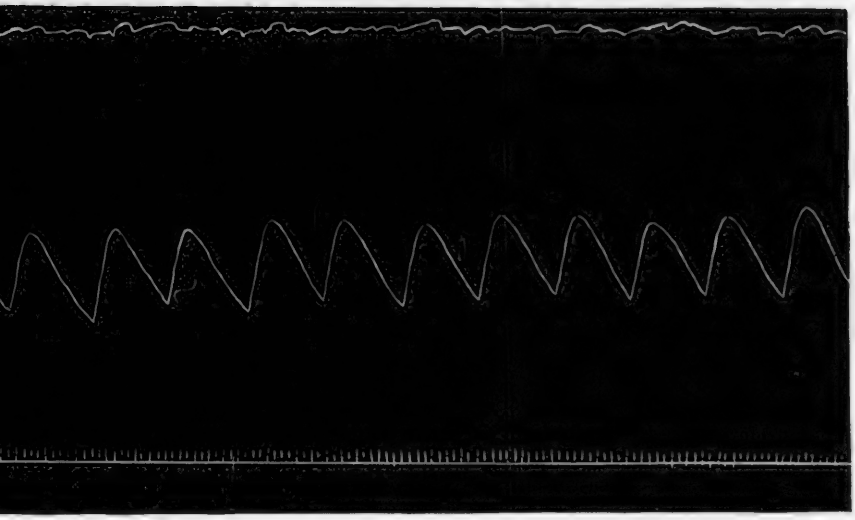


FIG. 26

PLATE IV.





U of M

THE EFFECT OF CHANGES IN TEMPERATURE UPON THE VISCOSITY OF THE "LIVING" BLOOD.[1]

By R. BURTON-OPITZ.

(From the Physiological Laboratory of Columbia University, at the College of Physicians and Surgeons, New York.)

It has been shown in an earlier paper of this series [2] that the viscosity of defibrinated blood can be changed by varying the temperature. While a rise in the temperature produces a decrease, a fall in the temperature causes an increase of the "molecular" friction of the blood. The question now arises, does the viscosity of the "living" blood vary in a similar manner, or better, is it possible to alter the viscosity of the circulating blood by changing the temperature of the surrounding medium?

To solve this question I performed a series of experiments upon dogs in accordance with the method described by Hürthle.[3] By means of this method it is possible to obtain a number of factors which are made use of subsequently in determining K, the coefficient of the viscosity of the blood. The factors involved in this calculation are the quantity of blood collected, during a definite period, the blood pressure, the specific gravity of the blood, and the length and internal diameter of the capillary-tube. The coefficients expressing the viscosity of the blood, are then compared with the coefficient of distilled water at 37° C., which, according to Poiseuille, possesses the value 4700. Hence, the viscosity of the blood is denoted in terms of multiples of the viscosity of distilled water at 37° C.

Only one capillary-tube was used in these experiments; its internal diameter measured 0.6636 mm. and its length 213.0 mm.

[1] This study was conducted under a grant from the Rockefeller Institute for Medical Research.

[2] Burton-Opitz, R., *Archiv für die ges. Physiol.*, 1900, lxxxii, 468.

[3] Hürthle, K., *Archiv für die ges. Physiol.*, 1900, lxxxii, 415.

The accuracy of the tube was tested by a series of preliminary experiments with distilled water.

The experiments were performed upon dogs during uniform light ether narcosis, no morphine having been administered beforehand. I tested the effect of warm and cold water baths and of hot dry air.

A.—THE EFFECT OF WARM AND COLD BATHS.

Six experiments in all were made, three with warm (43° C.) and three with cold water (23° C.). After securing the animal in a bath-tub, the normal viscosity of the blood was tested by three determinations at room temperature. The tub was then filled with warm or cold water. As the carotids were needed in experimentation, the ventral half of the neck and head were allowed to project above the surface of the water. Three other determinations of the viscosity were made fifteen, thirty, and forty-five minutes after the beginning of the bath.

The results of these experiments are compiled in Tables I and II; the first contains the experiments with warm, and the second, those with cold water. Each experiment is again subdivided into two parts, showing, on the one hand, the determinations of the normal viscosity, and, on the other, the determinations of the viscosity as influenced by the warm or cold bath. Among other details the tables also give the rectal temperature of the animal at the beginning and end of the experiment and the specific gravity of the blood. The latter factor was determined several times in the course of each experiment. Small pycnometers were used for this purpose.

These experiments prove conclusively that the viscosity of the blood reacts very sharply toward changes in the temperature of the surrounding medium. We observe that, in the case of the warm water, the coefficients become numerically larger than normal, while, in the case of the cold water, the coefficients become smaller. This implies that *the viscosity is decreased by warm and increased by cold water baths.* Let us take, for example, Experiment 2 of Table I. The viscosity of the blood ($K=862$) is in this case 5.4 times as great as that of distilled

TABLE I.

THE EFFECT OF WARM WATER BATHS.

Number of Experiment and Weight of Dog.		Determination Number.	Temperature °C. of water.	Temperature °C. of animal, rectal.	Time Elapsed since Beginning of Bath. Min.	Specific Gravity of the Blood.	Quantity Escaped from Capillary-Tube. Mgr.	Time. Sec.	Pressure Mm. Hg.	Viscosity Coefficient, K.	Mean Value of K.	Greatest Difference.
Exp. I. 11 kg.	Normal.....	1	—	37.2	—	1.05805	1688.3	13.04	168.60	796.71	788.47	74 43
		2	—	"	—	"	1780.7	14.43	163.40	787.50		
		3	—	37.0	—	"	1530.8	12.05	169.20	781.21		
	During Bath	4	43.0	—	15	1.05802	1667.4	13.47	153.92	834.88	846.09	
		5	"	—	30	1.05690	2039.2	15.08	168.00	840.50		
		6	"	41.3	45	"	2417.3	14.96	194.60	862.90		
Exp. II. 11 kg.	Normal.....	1	—	37.4	—	1.05355	1594.9	11.48	164.40	881.00	862.81	119.98
		2	—	"	—	"	1687.3	12.44	165.60	853.87		
		3	—	37.1	—	"	1768.9	12.62	171.20	853.56		
	During Bath	4	43.5	—	15	1.05260	1573.6	11.37	155.80	926.92	954.61	
		5	"	—	30	1.05202	1886.5	12.20	169.20	954.14		
		6	"	42.0	45	"	2346.5	14.90	167.30	982.79		
Exp. III. 8 kg.	Normal.....	1	—	37.5	—	1.0500	2004.2	13.28	151.90	1039.32	1038.09	101.39
		2	—	"	—	"	1782.1	11.89	149.60	1048.00		
		3	—	37.2	—	"	1663.1	12.32	137.50	1026.95		
	During Bath	4	42.0	—	15	—	1782.9	10.39	159.60	1126.48	1134.11	
		5	"	—	30	1.04832	1768.9	10.78	151.30	1136.38		
		6	"	39.8	45	"	2038.7	12.26	152.90	1139.48		

TABLE II.

THE EFFECT OF COLD WATER BATHS.

Number of Experiment and Weight of Dog.		Determination Number.	Temperature °C. of water.	Temperature °C. of animal.	Time Elapsed since Beginning of Bath. Min.	Specific Gravity of Blood.	Quantity Escaped from Capillary-Tube. Mgr.	Time. Sec.	Pressure Mm. Hg.	Viscosity Coefficient, K.	Mean Value of K.	Greatest Difference.
Exp. I. 10 kg.	Normal.....	1	—	38.0	—	1.06290	1183.4	9.65	170.60	742.82	721.31	87.46
		2	—	"	—	"	1536.0	10.76	204.20	722.80		
		3	—	37.0	—	"	1502.4	12.19	193.30	698.33		
	During Bath	4	22.5	—	15	1.06495	1761.3	13.77	198.00	666.27	647.53	
		5	"	—	30	1.06570	1907.5	15.33	199.60	642.49		
		6	"	32.4	45	"	2043.7	18.38	180.80	633.85		
Exp. II. 12 kg.	Normal.....	1	—	36.0	—	1.06030	1620.7	12.28	163.50	836.20	832.00	114.63
		2	—	"	—	"	2341.0	14.80	199.00	823.40		
		3	—	36.9	—	"	1860.3	13.69	168.30	836.40		
	During Bath	4	23.0	—	15	1.06065	1859.0	16.08	157.50	760.10	741.72	
		5	"	—	30	1.06238	1806.1	14.76	169.20	747.70		
		6	"	33.0	45	"	2069.3	17.42	171.20	717.37		
Exp. III. 12 kg.	Normal.....	1	—	37.0	—	1.05236	1367.7	12.90	113.30	976.70	978.02	129.17
		2	—	"	—	"	1742.1	13.62	136.50	978.01		
		3	—	37.0	—	"	1871.7	13.92	143.30	979.36		
	During Bath	4	22.0	—	15	1.05375	1761.9	14.20	135.60	953.80	898.32	
		5	"	—	30	"	2025.7	17.17	136.00	904.22		
		6	"	—	45	"	2382.6	17.61	150.10	886.41		
		7	"	33.5	60	"	1198.3	11.17	131.70	848.85		

water at 37° C. (K=4700). Under the influence of the warm water bath, the viscosity was reduced in the course of forty-five minutes (K=982) to only 4.7 times as great as that of distilled water at 37° C. Equally pronounced effects were obtained with cold water. In Experiment 3, Table II, the viscosity of the blood was increased in the course of forty-five minutes from 4.8 to 5.5 times as great as that of distilled water at 37° C. Moreover, when a comparison is made between the time and the total increase or decrease in the viscosity, it is seen that by far the greatest change takes place during the first ten to fifteen minutes after the beginning of the bath.

The specific gravity of the blood pursued in these experiments a course parallel to the viscosity. Warm water baths produced a decrease and cold water baths an increase in the specific gravity. The changes were very pronounced in both cases.

B.—THE EFFECT OF HOT AIR BATHS.

The method followed in the three experiments of this group was similar to that described in an earlier paragraph of this paper. The animal was placed in a glass case, through which a constant stream of dry hot air could be passed. As the carotids were used in experimentation, it was necessary to keep the head and neck of the animal outside of the case. As in the preceding six experiments, the animal was allowed to pant freely.

The normal viscosity of the blood having been determined several times, the temperature of the air in the case was gradually raised to about 60° C. Three determinations were then made, fifteen, thirty, and forty-five minutes afterward.

The results of the experiments are given in Table III. Each experiment is divided into two parts, showing the viscosity of the blood before, as well as during the air bath. The table also contains the temperature of the animal at different periods of the experiment, the temperature of the air, and the specific gravity of the blood.

A comparison of the coefficients proves that hot air baths do not render the blood less viscous, as one might suppose, but more

TABLE III.

EFFECT OF HOT AIR BATH.

Number of Experiment and Weight of Dog.		Determination Number.	Temperature ° C. of air.	Temperature ° C. of animal.	Time Elapsed since Beginning of Bath. Min.	Specific Gravity of Blood.	Quantity Escaped from Capillary-Tube. Mgr.	Time. Sec.	Pressure. Mm. Hg.	Viscosity Coefficient. K.	Mean Value of K.	Greatest Difference.
Exp. I. 9 kg.	Normal	1	—	37.2	—	1.06118	1475.3	12.17	160.70	780.80	769.07	22.07
		2	—	"	—	"	1492.2	12.87	156.70	765.85		
		3	—	37.1	—	"	1588.3	13.22	163.50	760.58		
	During Bath	4	65.0	—	15	1.06222	1448.2	12.67	155.80	758.58	751.74	
		5	"	—	30	"	1335.7	12.61	146.10	749.66		
		6	"	41.0	45	1.06480	1119.5	11.40	135.60	747.00		
Exp. II. 10 kg.	Normal	1	—	37.0	—	1.05338	1466.3	12.63	127.90	946.46	930.51	42.41
		2	—	"	—	"	1481.7	13.64	123.00	920.88		
		3	—	36.8	—	"	1574.9	13.28	133.80	924.19		
	During Bath	4	70.0	—	15	1.05520	1626.5	12.26	153.70	808.45	891.85	
		5	"	—	30	"	1530.8	13.15	137.30	889.00		
		6	"	42.2	45	"	1053.6	12.77	96.70	888.10		
Exp. III. 9 kg.	Normal	1	—	37.2	—	1.05435	2017.6	14.28	152.90	962.64	975.44	54.89
		2	—	"	—	"	1950.5	14.71	142.00	972.75		
		3	—	37.0	—	"	2293.7	13.17	183.10	990.95		
	During Bath	4	60.0	—	15	1.05500	2056.2	13.27	171.10	942.81	931.72	
		5	"	—	30	"	1802.6	13.61	148.00	931.81		
		6	"	40.5	45	"	1811.5	14.53	141.00	920.55		

so. Hence, the hot air and warm water produce directly antagonistic results. It seems, however, that the former is less powerful, because the increase in the viscosity produced by a constant exposure of the body to air of about 60° C. did not amount to more than fifty-five points in any of the experiments. For example, in Experiment 3, of Table III, the normal viscosity was 4.8 times as great as that of distilled water at 37° C. Under the influence of the hot air it became, in the course of forty-five minutes, 5.1 times as great. We will remember that the differences obtained with warm and cold water were much more marked and rapid.

The specific gravity of the blood became greater during the hot air bath. The viscosity and specific gravity preserved, therefore, a direct relationship with one another.

The coefficients for the normal blood of the dog vary in the present nine experiments between 1038 and 721, the average being 877. The viscosity of the dog's blood is therefore 5.3 times as great as that of distilled water at 37° C.

EXPERIMENTAL CIRRHOSIS OF THE LIVER.*

By RICHARD M. PEARCE, M.D.
(*From the Bender Laboratory, Albany, N.Y.*)

PLATE V.

The experimental studies upon which this communication is based were suggested by an investigation [1] of the necroses occurring in the liver of the dog as the result of the intravenous injection of hæmolytic immune sera. These necrotic lesions, which appear to have a very definite relation to thrombi composed of fused red blood corpuscles, vary in position and extent according to the amount of serum administered. Small doses cause focal lesions more or less isolated and irregularly distributed; large doses produce a diffuse necrosis which spares only the tissue about the larger portal spaces. The uniformity and extent of this latter lesion suggested the importance of studying the repair process which naturally follows it in those animals surviving the acute toxic effects of the serum. It was evident that the extent of the injury was such as to preclude complete repair by regeneration of liver cells alone. If, then, the defect were to be repaired by connective-tissue proliferation the resulting histological picture would, except for a difference in the distribution of the new tissue, closely resemble cirrhosis in man.

Experiments based on this method have not, heretofore, been made. Joannovics,[2] alone of the few investigators who have studied the lesions of the liver due to hæmolytic sera, has described repair about foci of necrosis. Flexner,[3] in 1894, while studying the lesions occurring in the organs of the rabbit after the injection of serum of the dog, observed a well-marked cirrhosis due apparently to repair about multiple necroses. The lesion was found, however, but once, and could not be repro-

* This study has been conducted under a grant from the Rockefeller Institute for Medical Research. Read before the American Association of Pathologists and Bacteriologists in Chicago, April 19, 1905.

duced in other animals. The literature contains, as far as I am aware, no other references to similar lesions due to serum injections.

Methods.—Dogs were injected either in the smaller branches of the femoral vein or in the abdominal cavity with serum obtained from rabbits which had received repeated injections of the red blood corpuscles of the dog. The dose usually employed was one cubic centimeter of serum to from 500 to 1000 grammes of body weight. The majority of the animals died within forty-eight hours; those which survived were killed at intervals varying from forty-eight hours to thirty-six days.

Character of the Primary Necrosis.—The necrotic lesions in the liver vary according to the dose of the serum. As the etiology and the general character of the focal necroses have been discussed elsewhere, only the more extensive form of necrosis, which through an equally widespread process of repair leads to cirrhosis, will be considered at this time. The macroscopic appearance of the liver in animals dying within a few minutes or hours after the injection of serum is that of intense congestion. Microscopically, the vessels are found to be distended by closely massed red blood corpuscles. The coalescence of red cells is very evident in the portal veins, while in the capillaries the occlusion leads to a distension so great that the columns of liver cells are more or less obscured. This congestion is less evident about the larger portal spaces. After twenty-four to forty-eight hours, the liver presents a uniformly mottled appearance; fine irregular yellowish brown or grayish yellow non-elevated areas being sharply separated from deeply congested or occasionally even hæmorrhagic areas. On section the brownish portions have a distinct hyaline appearance. The superficial portions of the liver are uniformly more extensively involved than are the deeper portions, though in some of the smaller lobes the necrosis may be quite general. Histologically, the necrosis, which is hyaline in character, involves all portions of the liver tissue except circular areas of varying size in the immediate neighborhood of the larger portal spaces (see Fig. 1).

In the necrotic areas the destruction, as far as the hepatic cells

are concerned, is uniform and complete; the cells of the capillaries may, however, persist. A narrow ring of liver cells with vacuolated protoplasm and pyknotic nuclei separates, as a rule, the necrotic from the normal tissue. Leucocytic infiltration may or may not be present. The capillaries of the necrotic tissue are widely dilated and tightly packed with swollen and distorted red blood corpuscles which may occur in masses or may retain distinct outlines. In the congested portal veins, on the other hand, the fusion is quite constant, the plugs for the greater part having a distinctly hyaline appearance. The perivascular spaces of the capillaries are distended with serum, and the bile passages of all sizes are, as a rule, dilated and engorged with bile.

The cause of the necrosis is an obstructive congestion of the capillaries and the smaller branches of the portal vein by fused masses of red cells. To the effect of this obstruction, essentially a thrombosis, is added the pressure exerted by the perivascular œdema and the over-filled bile capillaries. In parts of the liver midway between the larger portal spaces these factors are sufficient to overcome the pressure in the capillaries from the hepatic artery, and necrosis results; the cells in the neighborhood of the larger portal spaces, on the other hand, are preserved through a better blood supply dependent, presumably, upon the greater arterial pressure in this region.

A review of the literature of infarction of the liver in man and of experimental infarction shows no condition analogous to that here described; nor are any of the theories put forth concerning the etiology of infarction applicable to it. A possible exception is the widespread hæmorrhagic infarction produced by Wooldridge [4] as the result of injecting into the jugular vein of the dog a complex proteid substance derived from the thymus and other glandular organs. The infarctions were associated with thrombi in the branches of the portal vein, and in this regard, as well as in the character of the necrosis, Wooldridge's lesion resembles that just described. Moreover, in animals which survived fourteen days, he observed scattered foci of repair resembling early cirrhosis. He does not consider the

thrombosis alone to be sufficient cause for the necrosis, but believes that an important adjuvant is some change in the chemical composition of the blood caused by the proteid substance injected.

*The Reparative Process.**—Only fifteen of forty-three animals survived the acute effects of the serum a sufficient length of time to allow repair of the liver lesion to take place. The liver of each, however, shows some stage of repair, and as various periods of from thirty-eight hours to thirty-six days are represented, it has been possible to study all stages of the development of the cirrhotic lesion.

At thirty hours the first and only evidence of repair is karyokinesis of the liver cells. This, however, is not conspicuous and the mitotic figures are found only after prolonged search. They occur, uniformly, not at the edge of the necrotic tissue but in liver cells two or three rows removed. Mitotic figures have never been seen earlier than thirty-eight hours, and at this period in but one animal.

The period from forty-eight to sixty hours is represented by five animals. The character of the repair is the same in all, but some difference in degree is evident. The most striking feature is the proliferation of the endothelial cells. These surround the necrotic tissue and penetrate it from every side. Similar cells, corresponding to the remains of the capillaries, may be seen in some places within the necrotic areas. Many of these cells have irregular nuclei suggestive of mitosis, but a definite karyokinetic figure, in a cell surely endothelial, was found at this stage but once; this was in the liver of an animal killed exactly forty-eight hours after injection. The endothelial cells show a tendency to surround small fragments of necrotic liver cells, and occasionally they contain one or two red blood corpuscles. Leucocytic infiltration occurs to a very slight extent.

By the end of the fourth day the proliferation has advanced so rapidly that the bulk of the necrotic tissue has been replaced.

* Only a general outline of the reparative process will be given at this time; the details of the finer changes and their interpretation will be presented in a future communication on the repair of liver tissue.

Within the necrotic areas the bands of new tissue are more definite. Mitotic figures in liver cells are so numerous that three or four may be found in one field of a one-twelfth lens. A few lymphoid cells are seen in the portal spaces.

The picture at the fifth day differs only in the extent of the process. The new tissue has assumed all the characteristics of granulation tissue and contains giant cells which surround fragments of hyaline material. The first suggestion of intercellular fibrillæ is seen at this stage.

The later stages, from the eleventh to the thirty-sixth day, illustrate the gradual transformation of this young granulation tissue into a very definite connective tissue. At the eleventh day the new blood-vessels are very prominent and the fibrillated character of the connective tissue well marked. All stages after the fourth day show a few scattered lymphoid cells, but this has not been a prominent feature except in one animal, killed on the twenty-fifth day, in which the tissues of the portal spaces are filled with lymphoid and plasma cells.

The oldest as well as the most typical lesion was that found in an animal killed on the thirty-sixth day (see Fig. 2). Macroscopically, the liver was much firmer than normal, had a finely granular surface, and was deeply bile stained. The capsule showed irregular areas of thickening and was mottled by an ill-defined congestion. On section, a distinct pseudo-lobulation was evident, definite islands of brownish yellow liver tissue being marked off by a fine grayish network of newly formed tissue. Histological examination shows broad bands of connective tissue entirely replacing the necrotic areas and forming a uniformly arranged network separating the surviving islands of liver tissue about the larger portal spaces. The new tissue is distinctly fibrous and contains many newly formed blood-vessels and bile ducts; it stains deeply by Mallory's method for connective tissue. The formation of new liver cells is still demonstrable. Fragments of necrotic hyaline cells, not infrequently partially calcified, are found in the midst of the new connective tissue. Large irregular multinucleated masses of protoplasm surround these and are apparently active in englobing and removing them.

These multinucleated cells, essentially foreign body giant cells, are derived in part from endothelial cells and in part from liver cells. Associated with them are numerous multinucleated liver cells without inclusions. Lymphoid and plasma cells are present, but only in small numbers.

Discussion.—The final stage of the lesion herein described, constituting as it does a chronic interstitial hepatitis of diffuse but uniform distribution, may justly be termed an experimental cirrhosis. No close analogy, however, can be drawn between it and human cirrhosis, for, with the possible exception of the so-called central cirrhosis associated with chronic passive congestion, a form of interstitial hepatitis with similar distribution of the new tissue does not occur in man. In this connection Bostroem's [5] recent discussion of liver cirrhosis as a repair process in chronic passive congestion is of especial interest. As the result of a study of material from man he reaches the conclusion that the congestion causes destruction of liver cells, not necessarily by pressure of the widened capillaries, but through nutritive and functional disturbances. With these conditions are associated rupture of the capillary wall and the escape of red cells into the perivascular lymph spaces. Thrombosis also is not an infrequent occurrence and has a close relation to the destruction of liver cells. The repair, which is most active in the region of the portal spaces, results in a very definite cirrhosis. When one considers that the necrosis in my experimental lesion is preceded by an intense congestion associated with all the etiological factors described by Bostroem, the analogy of the repair lesion to the cirrhosis which he describes becomes very apparent. Comparisons as to the etiology of cirrhosis in general are not justifiable, for we know of no toxic substances associated with abnormal conditions in man which are capable of causing the widespread necrosis seen in these experimental lesions. The only condition comparable is acute yellow atrophy with which the early necrotic and reparative lesions of the second to the sixth day have much in common.

On the other hand, however, these experimental lesions demonstrate that a cirrhosis may follow extensive primary destruc-

tive lesions, and thus support the theory of Kretz [6] that cirrhosis is essentially a reparative process. Kretz, who bases his conclusions on an extensive study of the disease in man, believes cirrhosis to be the result of successive processes of repair following repeated focal injuries of the liver parenchyma. The primary lesion is, he thinks, a destruction of groups of liver cells at the periphery of the lobule. After such destruction the uninjured cells of the lobule, as well as the cells of the bile ducts proliferate in an attempt to repair the injury. The continuous occurrence of degeneration and regeneration causes the formation of new connective tissue which eventually atrophies, thus leading to the picture seen at autopsy. Cirrhosis, therefore, is the result of repeated localized destructive lesions from which the liver has more or less recovered. This view, it will readily be seen, accepts Weigert's [7] opinion that the primary lesion in all interstitial new growth of tissue is cell death, and also includes Kirikow's [8] opinion that in addition to cell death the continual action of the toxic substance is necessary; but adds the important factor of continuous regeneration.

W. G. MacCallum's [9] study of the regenerative changes in human cirrhosis strongly supports the theory of Kretz, as do also the studies by McPhedran and A. B. MacCallum,[10] Meder,[11] Marchand,[12] Stroebe,[13] Barbacci,[14] W. G. MacCallum,[15] and others of repair in acute yellow atrophy of the liver.

The experimental studies of repair after various mechanical injuries also indicate the ease and rapidity with which liver parenchyma repairs loss of substance. From the observations of Podwyssozki [16] and Ponfick [17] it is seen that slight injuries are repaired by the proliferation of either liver cells or the smaller bile ducts, connective-tissue repair occurring only when loss of substance exists. Of special interest is the true hyperplasia observed by Ponfick and by v. Meister [18] after extirpation of large portions (one-quarter to three-quarters) of the entire liver.

On the other hand, the argument is presented that focal necrotic lesions of the liver frequently occur in man without repair either by proliferation of parenchymatous cells or by

formation of connective tissue. In this connection the hepatic lesions of typhoid fever, diphtheria, and other acute infections, as well as various experimental infections of animals, are quoted. It is probable, however, that in such cases the reparative power of the cells is in some way delayed, or consists possibly of a true regeneration of liver cells without the proliferation of connective tissue; for in hog cholera, in which, above all other diseases, necroses of the liver are most abundant and widespread, cirrhosis of the liver commonly occurs. Salmon, Smith, and Kilborne,[19] in the study of hog cholera in an epidemic including both acute and chronic cases, found well-marked cirrhosis in about one-half of the animals examined.

The question as to whether the interstitial tissue or the parenchyma is primarily affected in cirrhosis receives little enlightenment from the literature of experimental cirrhosis. Heukelom [20] who reviewed the literature up to 1896 collected nineteen different methods said to be capable of producing cirrhosis, and to these Joannovics [21] in a recent summary adds others. None, however, produces a lesion analogous to that occurring in man. These methods may readily be divided into two groups. In the first we have procedures essentially mechanical in nature, as ligation of the ducts or vessels of the liver, the injection of irritating substances into the ducts or directly into the liver substance, and their application to its surface. The second group includes the administration by the mouth, or by subcutaneous or intravenous injection, of various toxic substances. As a general rule, the mechanical methods lead to a more or less irregular connective-tissue formation, sometimes with considerable sclerosis, but the lesion under these circumstances is more of an atrophy than a cirrhosis. The toxic agents, for which positive results have been claimed, have been introduced so that they reach the liver through the blood stream. The lesion attributed to such substances is usually described as a primary degeneration of the liver cells with a slight increase of connective tissue limited, as a rule, to the portal spaces; in a few instances a definite perilobular growth of connective tissue, independent of parenchymatous degeneration, has been described. Few of these lesions,

however, even in their final stages, are comparable to the cirrhosis of man, and in none has it been possible to follow step by step the development of a lesion which could properly be termed an experimental cirrhosis.

Summary.—The reparative process which follows the widespread necrosis of the dog's liver caused by the injection of hæmagglutinative serum constitutes a chronic interstitial hepatitis of definite and constant character. This is not only a new type of experimental hepatic lesion, but is more definitely a cirrhosis than is any other experimental lesion hitherto described. It is of importance in explaining the histogenesis of cirrhosis, and incidentally various repair processes in the liver; but it does not aid in the elucidation of the etiology of cirrhosis in man, nor does it explain the peculiar arrangement of the new connective tissue in any form of human cirrhosis except possibly that associated with chronic passive congestion. It definitely demonstrates, however, that cirrhosis may follow extensive primary destructive lesions, a view not yet fully accepted, and supports the contention of Kretz that cirrhosis is essentially a reparative process.

EXPLANATION OF PLATE V.

Fig. 1.—The primary lesion. Extensive hyaline necrosis sparing only the tissues about the larger portal spaces. Thirty-eight hours.

Dose 1 : 1000. Methylene blue and eosin. No. 2 oc., No. 3 obj.—Leitz.

Fig. 2.—Experimental cirrhosis. Broad bands of newly-formed connective tissue surround the islands of normal liver about the portal spaces. In the stroma may be seen newly-formed bile ducts and numerous giant cells. Thirty-six days

Dose 1 : 1000. Hæmatoxylin and eosin. No. 3 oc., No. 3 obj.—Leitz.

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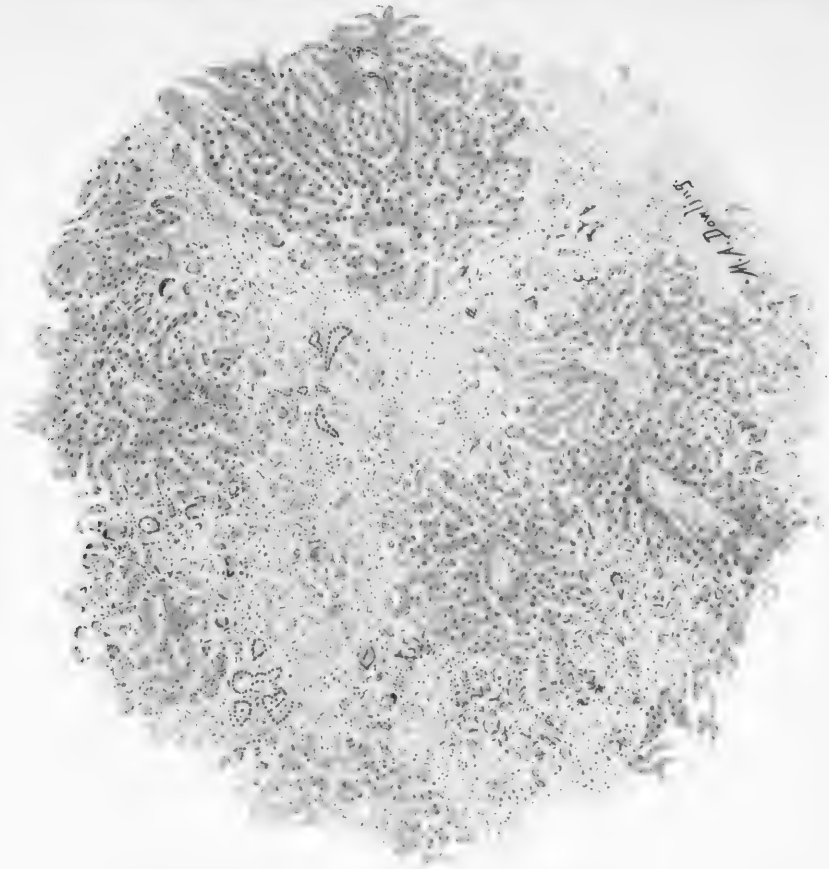

FIG. 2

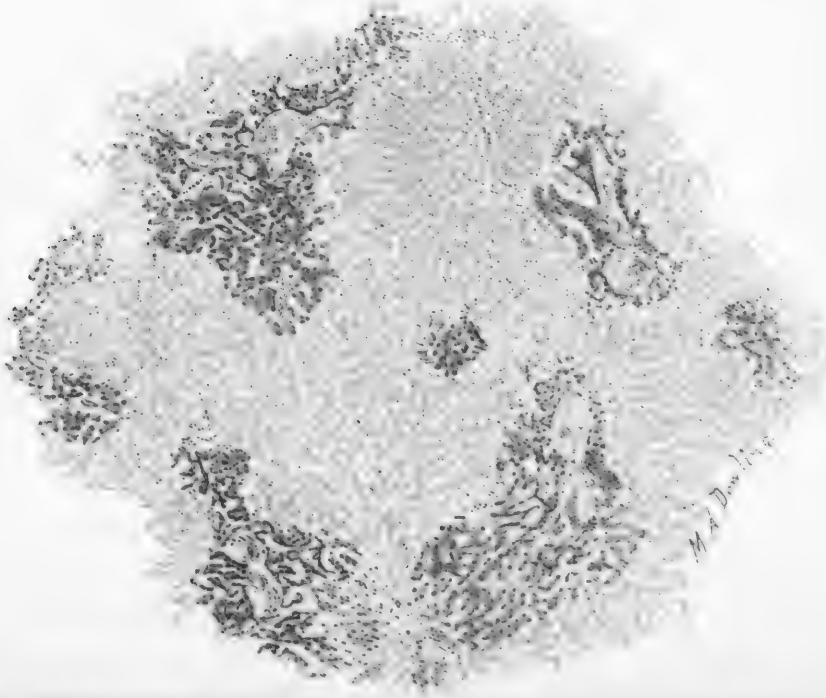

FIG. 1

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EXPERIMENTAL ARTERIOSCLEROSIS.*

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Plates VI and VII.

The complexity of the pathological picture in the arteriosclerosis of man and the difficulty of determining the nature and sequence of the early changes have led many investigators to attempt the experimental production of the disease in the hope of thus solving some of its many confusing problems. Until within the last two years all such efforts have been unsuccessful. It is true, that Thoma described a diffuse arteriosclerosis in the dog as the result of a chronic experimental aortic insufficiency, and that Gilbert and Lion [1] produced scattered sclerotic and calcareous changes in the vessels of animals by injecting bacteria and their toxins. Thickening of the vascular walls has been described also as the result of poisoning animals with lead. These results, however, have been inconstant and lack confirmation. Jores,[2] in his excellent monograph on arteriosclerosis, published in 1903, reached the conclusion, based on a critical review of the literature, that all experimental methods fail to cause lesions similar to those occurring in man. Various endarterial lesions, obliterative or otherwise, as well as diffuse inflammation and atrophy, have been produced by injections of bacteria or their toxins, by the application of irritating substances to the perivascular tissues, and by ligation and other forms of mechanical injury; but none of these lesions is analogous to true arteriosclerosis.

It is a curious coincidence that in the same year in which Jores reached this conclusion, Josué [3] described experimental lesions

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in the aorta of rabbits, somewhat similar to those of human arteriosclerosis. These were caused by frequent intravenous injections of adrenalin. With the exception of a few inconclusive experiments of Jores, who gave dogs adrenal tablets in their food in the hope of obtaining lesions due to heightened blood-pressure, Josué appears to be the first investigator to attempt the experimental production of arteriosclerosis by the use of the principle of the adrenal gland. His results have been confirmed by Erb,[4] Rzentkowski,[5] Fisher,[6] and others. Their reports include but a small group of experiments and deal for the most part with the late changes, which they describe variously as arteriosclerosis, atheroma, and calcification. The principal histological changes to which they call attention are alteration in the elastica and the infiltration of lime salts.

The importance of a method capable of producing an experimental lesion so closely resembling human arteriosclerosis is apparent. It allows an opportunity to study many of the obscure problems of the disease, as the nature of the primary changes, the sequence of these changes, and the combinations which constitute the fully developed lesion. It was with the hope of elucidating some of these problems that the present investigation was undertaken. The results have been satisfactory beyond all expectation. Vascular changes capable of throwing much light upon the pathology of arteriosclerosis, and also that of aneurism, have been readily produced. Only a general outline of these will be given at this time, for many of the details of the histological changes, especially of the process of repair, are so important that they require a more extended study. A consideration of these details is therefore reserved for a future publication based on a second series of experiments now in progress.

Methods.—Rabbits have received in the veins of the ear repeated injections of a 1 to 1000 solution of adrenalin. The solution was prepared by a chemist with due regard to asepsis and chemical purity. Physiological salt solution was used as a medium. In addition to adrenalin (Parke, Davis, & Co.), it contained also chloretone in the proportion of one half of one

per cent., and a sufficient amount of hydrochloric acid to make the solution faintly acid.

A dose of three minims * repeated every other day has been the usual procedure, though in some experiments the dose has been gradually increased. Not infrequently the early injections cause death from acute dilatation of the heart and pulmonary œdema. Such accidents usually occur after any one of the first seven or eight injections. If the animal survives this period it appears to gain a certain amount of immunity, or at least tolerance to adrenalin, so that the dose may be gradually increased during several weeks until from twenty to twenty-five minims may be given every day. This increased resistance to adrenalin has been noted also by Erb and Fisher. The animals have been killed after periods varying from a few days to eight and a half weeks.

For histological study, tissues have been preserved in Zenker's fluid, alcohol, and six per cent. formalin, imbedded in celloidin, and stained with hæmatoxylin and eosin, and by Weigert's elastica and Mallory's connective-tissue methods. The Scharlach R. and osmic-acid methods have been employed for the demonstration of fat. Sections hardened in Flemming's solution and stained by hæmatoxylin have been found to be most satisfactory for purposes of general study. By this method, the nuclei and areas of calcification stain blue; fat, if present, black; the elastic fibres stand out prominently as light greenish-yellow glistening lines, while all other structures are of a faint gray color.

Results.—Of twenty animals receiving adrenalin, nine succumbed to the acute effects of the drug within fifteen days. These nine represent animals receiving one, three, four, five, seven, and eight injections of adrenalin on alternate days. In some instances death resulted within a few minutes after the injection; in others, after a few hours. The immediate effect of the

* Throughout this report the dose will be given in minims. It would be better perhaps to use the values of the metric system, but as Josué and others give the dose in minims or drops, we have for purposes of control used the same system of measurement.

intravenous administration of adrenalin is collapse with difficult and rapid respiration. The animal lies on its abdomen with legs outstretched and head resting on the table or raised in spasmodic respiratory effort; death, preceded by severe convulsive movements, may occur immediately. Other animals recover from the immediate manifestations only to succumb after a few hours. Upon post-mortem examination, the usual picture is acute dilatation of the heart and œdema of the lungs, with not infrequently small hæmorrhages beneath the pleura and pericardium; in one animal hæmorrhages were also found in the adrenal. These acute lesions indicate a very serious disturbance in the terminal vascular territories and are worthy perhaps of further investigation; but as our problem has to do only with the histological changes in the larger vessels its scope has been limited to that extent. The theoretical conclusions to be drawn from experimental adrenalin œdema and their possible application to acute œdema of the lung in man have recently been discussed by Josué.[7]

In but two of this group of nine animals were changes in the vessels demonstrable. These will be discussed later.

The second group of eleven animals represents those receiving from eight to twenty-eight injections during periods varying from sixteen to fifty-nine days. One of these died on the twenty-fifth day from spinal hæmorrhage; another on the forty-sixth day, from unknown cause, three weeks after the cessation of injections; the remaining ten were chloroformed. The aorta in six of these showed marked gross lesions; in the other five it was negative both macroscopically and microscopically. It is of interest that these five animals were all from the same litter and each weighed about 750 grammes at the time of the first injection. Pic and Bonnamour[8] have come to the conclusion that it is impossible to produce vascular lesions by adrenalin in rabbits weighing less than 2000 grammes, and quote in support of this opinion their negative experiments with animals weighing about 1200 grammes. That this is not an absolute rule is shown by the fact that the most characteristic and advanced lesions in our series occurred in an animal weighing 870 grammes at the

beginning and 1020 at the end of the experiment. Otherwise, however, our experience with small animals supports the contention of Pic and Bonnamour.

Attempts to produce lesions in dogs have been unsuccessful. Both old and young dogs have been used. One of the latter, a puppy 2120 grammes in weight, received in the ear vein in one month one hundred and thirty minims of adrenalin in doses rising gradually from four to twenty minims. Although respiratory and cardiac disturbance frequently occurred immediately after injection, thus indicating a definite physiological action of the adrenalin, no evidence of vascular lesions could be found at autopsy or upon microscopic examination.

Gross Lesions.—These appear to be limited to the aorta; the involvement of other arteries as the brachial, carotid, and renal, which Erb describes, we have not found. The lesions in the aorta are most marked in the thoracic portion and are seldom found below the cœliac axis or in the first portion of the arch. The earliest change in the vessel wall manifest to the naked eye is a faint longitudinal or irregular grayish streaking of the intima without thickening. This appearance was seen as early as the ninth day in a rabbit which had received five injections. After eight to fifteen injections, especially if the animal is allowed to live for a week or longer after the last injection, very definite lesions are apparent. These consist of irregular, isolated, or confluent areas, usually slightly depressed, of a pearly gray color, and almost constantly calcified. The following protocol illustrates this condition:

Rabbit No. 11.—Weight, 2300 grammes. Killed May 2 after injections as follows: March 29 and 31, 4 minims; April 2 and 4, 5 minims; April 6 and 8, 7 minims; April 10, 10 minims; April 12, 12 minims; April 14, 15, and 16, 15 minims. *Autopsy.*—The aorta is distinctly dilated, measuring twelve millimetres in circumference at the arch. Beginning at the origin of the left carotid artery is an irregular patch, thirty-seven millimetres long, averaging two and a half millimetres in width, and extending in a spiral course along the aorta for a distance of thirty millimetres so as completely to encircle it. Along this patch the wall of the aorta is slightly dilated. The media is firm, parchment-like, and so distinctly calcified that the vessel cracks in several places when the arch of the aorta is straightened. At one side of this large patch are two very small oval areas of similar structure. There is no atheroma or ulceration. All the other organs are entirely normal.

The late lesions occurring after from twenty to twenty-five injections are very well marked. The aorta is more or less distorted, rigid, and non-elastic. Irregular dilatations alternate with elevated brittle areas of calcification. Distinct ulceration with atheroma is not readily demonstrable. Diffuse calcification is not infrequent and small aneurismal dilatations may be present. The following protocol illustrates the advanced lesions occurring in animals receiving large doses during a considerable period of time:

Rabbit No. 3.—Weight, 870 grammes. Received first injection of 3 minims in ear vein on November 30, 1904. Similar injections were repeated on alternate days until December 30, after which date the dose was rapidly increased until it reached 20 minims daily. The animal was killed on January 20, 1905. *Autopsy.*—Weight, 1150 grammes. The thoracic aorta measures six millimetres in diameter, is distinctly dilated, irregular in outline, and stiff and brittle, although the wall is apparently thinned. This condition extends as far as the cœliac axis, beyond which point the abdominal aorta is apparently normal. On opening the aorta the inner surface of the abdominal aorta above the cœliac axis shows numerous yellowish, slightly raised, calcified patches from one to one and a half millimetres in diameter, which in the upper part of the thoracic aorta become confluent. Except in one of the small patches situated in the upper portion of the abdominal aorta there is no evidence of ulceration. All other organs are normal.

The following description from the protocol of a parallel experiment illustrates the tendency to the formation of small aneurisms:

Rabbit No, 2.—The aorta averages three millimetres in diameter except at three places where distinct dilatations occur. The first of these begins three centimetres below the origin of the aorta and involves the right anterior wall of the aorta for a distance of two centimetres, giving the appearance of a fusiform aneurism; the diameter of the aorta at this point is four millimetres. A second dilatation, two and a half centimetres in length, occurs in the distal part of the thoracic aorta, and a third involves the abdominal aorta for a distance of one centimetre immediately below the cœliac axis. On section, the inner surface of the aorta shows sharply circumscribed saccular dilatations, varying from one to two millimetres in depth and corresponding to the protrusions described above. The walls of these aneurisms contain firm placques of calcareous material. The intervening aorta shows a number of yellowish-white elevated areas one to three millimetres in diameter. The aorta below the dilatation at the cœliac axis is macroscopically normal.

Histology.—In the group represented by the nine rabbits which died during the first two weeks, the vessels of but two

showed histological changes. These were animals which had received four and five injections respectively; the first dying on the fifth day and the second on the ninth day. Scattered through the middle coat of the aorta in each of these are small longitudinal or occasionally irregular, finely granular foci of degeneration. In these areas no nuclei are visible and the muscle fibres are transformed into a finely granular, almost hyaline material which stains deeply with eosin. The exact nature of this change is not readily determined, but as the various methods of demonstrating fatty transformation are negative, and those which differentiate elastic fibres show no change in the staining reaction of the latter, the condition would appear to be a simple necrosis limited to the muscle fibres. The elastic tissue has, however, undergone very characteristic morphological changes which are found only in the degenerated areas, and are due, presumably, to mechanical influences. In the normal aorta the elastic fibres have a distinctly wavy or curled appearance and are definitely separated from one another; in the areas described the fibres lose this appearance and become straightened and closely approximated. They are also swollen and occasionally appear to be fused together. Fracture of the fibres is seldom seen at this stage of the process.

A more diffuse lesion of this character was found in an animal killed on the twenty-fourth day after receiving eleven injections. The changes involve the entire circumference of the vessels, sparing to a certain extent the innermost and outermost portions of the media. In this central zone few or no nuclei can be found, and the altered tissue presents a uniform, finely granular appearance, relieved only by the glistening lines of the elastic fibres. The latter by selective stain are seen to be so closely massed together that individual fibres can be distinguished only with great difficulty. On either side of this zone the less degenerated portions of the media show irregular areas which take the hæmatoxylin stain in a manner very suggestive of early infiltration with lime salts. There is, however, no distinct calcification and no fracture of elastic fibres.

Lesions of great interest in comparison with the above were

observed in two rabbits which had received the same number of injections, but which were allowed to live for ten and eighteen days, respectively, after the last injection. In the aorta of each are found a few areas of granular necrosis, but for the most part these areas have been altered by infiltration with lime salts. From the topography of the areas of calcification and their relation to the necrotic foci, it is evident that the deposition of lime salts follows the necrosis. It would appear therefore that the primary degenerative lesions are well advanced by the end of the third week, and that after this but one or two weeks are sufficient for advanced calcification; the latter may, however, occur much earlier, for in one rabbit killed on the sixteenth day small but very definite foci of lime infiltration are present.

In the areas of lighter deposition of lime it is seen that these salts are deposited first between the elastic fibres, an observation which indicates that the destruction of the muscle fibre is the older, and therefore in all probability the primary, lesion. In the areas of advanced calcification, although the elastic fibres appear to be completely destroyed by the calcareous transformation, Weigert's stain shows them to be still present, though they stain poorly and are frequently fractured. Not uncommonly at a point of fracture one bundle of fibres overlaps another, the ends being splintered together by an encapsulating mass of lime salts. These fibres are always perfectly straight except at angles formed by fracture. A definite relation appears to exist between calcification and fracture of the elastic fibres. In areas of the most extensive calcification few breaks in the elastic fibres have been observed, while, on the other hand, they are constantly present in the aneursimal dilatations which show comparatively little calcification.

Lesions of this stage show the first evidence of repair. This is indicated by the collection of newly formed cells about the foci of calcification. These cells are closely massed, surrounded by but a slight ring of protoplasm, and appear to be of connective-tissue origin, though it has not always been possible to distinguish between such cells and the nuclei of smooth muscle fibres. No accumulation of polymorphonuclear leucocytes or lymphoid

cells has been seen. Of greater interest are the proliferative changes in the intima. These occur only opposite distinct breaks or considerable depressions in the media, and include proliferation of the lining endothelium and, to a greater extent, of the subendothelial tissues. The endothelial cells proliferate to form two or three layers of cells longitudinally arranged, while the subendothelial space, barely visible in the normal aorta, becomes very prominent owing to the presence of closely packed oval nuclei arranged vertically to the lining endothelium. Between the nuclei are delicate newly formed connective-tissue and elastic fibrils; in the late stages the newly formed elastic fibrils are very prominent. In the angle of fracture the nuclei of the media assume a perpendicular arrangement very striking in contrast to their longitudinal arrangement elsewhere. The entire picture is distinctly that of a compensatory proliferation: an effort to strengthen the weakened point in the vessel wall.

Such are the essential phases of this lesion in the order of their sequence. The more prolonged experiments offer no fundamentally new features; but the combinations of these primary changes in the course of the more extensive involvement of the vessel wall lead to a complex histological picture somewhat resembling advanced human arteriosclerosis. This is seen only in animals which have received gradually increasing doses during a period of seven or eight weeks. The vessel wall becomes greatly thickened, not only by infiltration of lime salts, but also by an extensive repair process which involves the intima as well as the media. Small foci of complete necrosis, analogous to atheroma and entirely different from the primary degeneration, are also seen in areas in which the elastic fibres are completely destroyed. Such foci present a uniform, finely granular appearance and stain deeply with eosin; the osmic-acid method for fat is negative, but a few fine droplets are evident after treatment with Scharlach R. About such areas repair takes place, but it is not as active as it is about the masses of lime salts.

The most striking feature of the late histological picture is the extent of repair in the intima. The latter, with its newly formed

connective-tissue and elastic fibrils, becomes so greatly thickened that it constitutes in some places from a quarter to a third of the entire vessel wall, and offers convincing evidence of the compensatory nature of the repair process.

It is in these late lesions that the small aneurisms are seen. They occur at points where the elastica is so completely destroyed as to be transformed into an indistinct mass of fractured granular and fused fibres. The transition from normal to necrotic fibres is always sharp and distinct and marked by complete fracture of all elastic tissue. It is worthy of note that in the thinned wall of these aneurisms the degree of calcification is, as a rule, less marked than elsewhere; the adventitia is compressed, but otherwise unaffected.

The changes in other organs include enlargement of the heart, œdema and congestion of the lungs, and occasionally degenerative changes in the heart and skeletal muscles. In one case degenerative lesions were found in a nerve ganglion adherent to the aorta. It was thought that these changes might have some etiological relation to the vascular lesion, but a thorough study of all our material failed to reveal lesions in other animals, or in other ganglia of the same animal. The affected ganglion was in the adventitial tissue of an aneurismal dilatation, and the effects of pressure and disturbed blood-supply were sufficient, apparently, to account for the changes observed.

The Mode of Action of Adrenalin.—The manner in which adrenalin produces these lesions is a matter of widely varying opinion and, unfortunately, one which cannot readily be determined by our method of experimentation. Until we know more about the toxic action of adrenalin and can distinguish between the lesions due to this action and those due to increased blood-pressure, and especially between its direct and secondary effect on the blood-vessels, we can hope for no elucidation of this problem. Drummond,[9] who has recently made a thorough study of the histological lesions caused by adrenalin, divides them into those due to toxic action and those due to increased blood-pressure. It is obvious that such a classification is difficult, for it is impossible to determine to what extent degenerative lesions

are due to interference with the circulation through action on the blood-vessels.

A discussion of the variety of ways in which adrenalin might act, directly or indirectly, to cause degenerative changes in the media of the aorta would, in the present state of our knowledge, be of little value. The difficulty of reaching definite conclusions is illustrated by the diversity of opinion expressed by those who have previously produced the lesions here described. Josué believes them to be due to a combination of a specific toxic influence and the increased blood-pressure; Rzentkowski believes in the latter influence only; Erb ascribes an important influence to disturbance of the *vasa vasorum;* Fisher thinks that, aside from the increased blood-pressure, disturbances of metabolism play an important part; Lissauer [10] believes that the toxic influence is the most important factor, but offers no explanation of its mode of action.

No definite conclusion can be drawn from our own experiments. The observation, that the earliest changes occur in the media and are apparently primary in the smooth muscle fibres, suggests, in view of the well-known physiologic action of adrenalin on this tissue, a direct and selective toxic action; but the absence of similar lesions in vessels other than the aorta does not support this view. For the same reason, and also because of the absence of changes in the adventitia, the theory concerning the *vasa vasorum* appears to be untenable. On the other hand, if we assume, as is very probable, that the changes in form and arrangement of the elastic fibres are due to the same factor or factors which cause the destruction of the muscle cells, and are not secondary to the latter, we have a strong argument in favor of the influence of a greatly heightened blood-pressure, for it is conceivable that these diffuse changes might result from a continued severe stretching of the vessel wall at the time of the ischæmia due to the primary action of the adrenalin. In other words, it is possible that at the period of vascular spasm produced by the adrenalin the nutrition of the vessel wall is altered. This alone would not explain the limitation of the lesion to the aorta, but the added mechanical disturbance due to the extreme disten-

tion of the vessel wall would appear to be sufficient to bring about a condition analogous to anæmic necrosis. These problems, however, must be settled by other methods of experimentation. Although it is impossible to determine to what extent the primary changes are mechanical and to what extent toxic in nature, it is certain that some of the secondary changes, as the fracture of elastic fibres and the formation of aneurisms, are largely due to mechanical factors.

Comparison of the Experimental Lesions and those Occurring in Man.—If due allowance is made for the difference in size between the aorta of the rabbit and that of man, it must be admitted that the lesions produced in the former, as the result of the administration of adrenalin, are somewhat similar to those occurring in human arteriosclerosis. They are not, however, analogous. All the essential processes are represented and, considering the delicate character of the wall of the rabbit's aorta, the lesions are relatively as extensive and cause the same degree of deformity. There is, however, as compared with the human lesion, a difference in the initial change and in the degree of atheroma, which is insignificant and limited to the media. Extensive atheroma, however, cannot be expected in a vessel wall as thin as the rabbit's aorta, and, moreover, in none of these experiments has a period of time sufficient for the occurrence of degeneration in areas of excessive intimal proliferation elapsed. More prolonged experiments will yield, it is to be hoped, lesions more conclusive in this respect. For the present the condition may perhaps be regarded as arteriosclerosis of the rabbit, but not as a condition analogous to the arteriosclerosis of man.

In the light of the information gained from the study of these experimental lesions, it would be desirable perhaps to discuss critically the various theories concerning the nature of arteriosclerosis and especially the character and sequence of the histological changes. Such discussion, however, does not come within the scope of this communication, and many minor points must be determined before comparisons are justifiable. It is sufficient, for the present, to point out the strong support afforded Thoma's view, that the primary lesion of arteriosclerosis occurs

in the media and is, essentially, the result of changes in the elastica; and that the alterations in the intima constitute a repair process the object of which is to compensate for the weakened media and the widened lumen.

EXPLANATION OF PLATES VI AND VII

Fig. 1.—Gross appearance of a portion of the aorta from an animal which had received twenty-seven injections and was killed on the twenty-ninth day. The drawing is twice the actual size of the original specimen.

Fig. 2.—Diffuse degeneration of the central zone of media; eleven injections; twenty-fourth day. Hæmatoxylin and eosin; No. 4 oc., No. 3 obj.—Leitz.

Fig. 3.—Microscopic picture of lesion pictured in Fig. 1. Destruction of the media with infiltration of lime salts; extensive proliferation of the intima. Hæmatoxylin and eosin; No. 4 oc., No. 3 obj.—Leitz.

Fig. 4.—Elastic tissue stain of an area very similar to the above, illustrating extreme destruction of the elastica.

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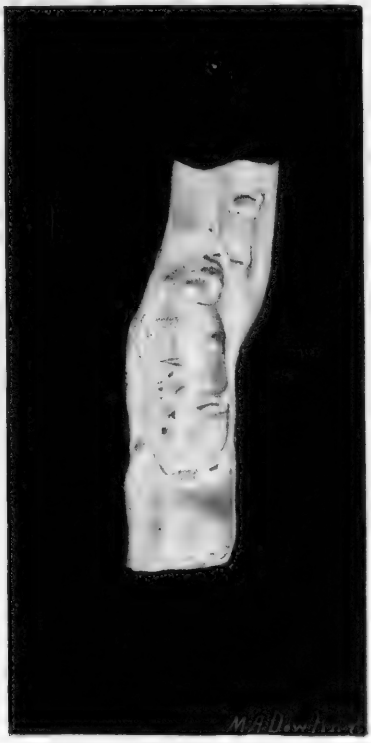

FIG. 1

FIG. 2

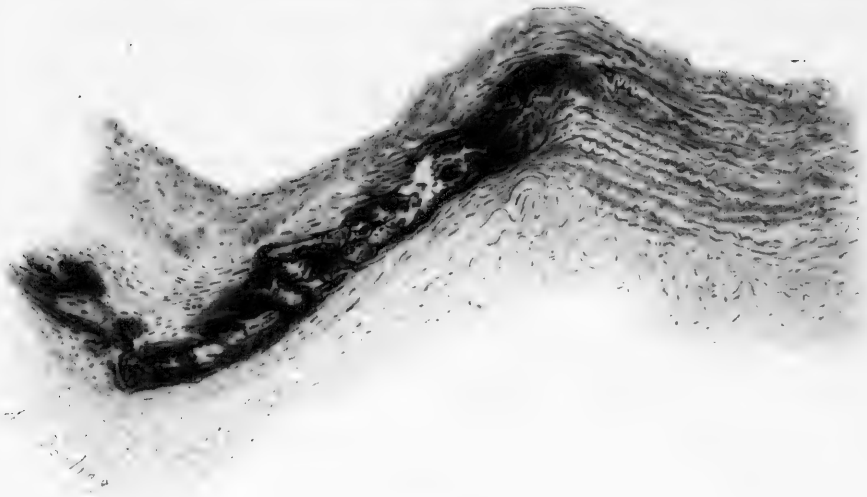

FIG. 3

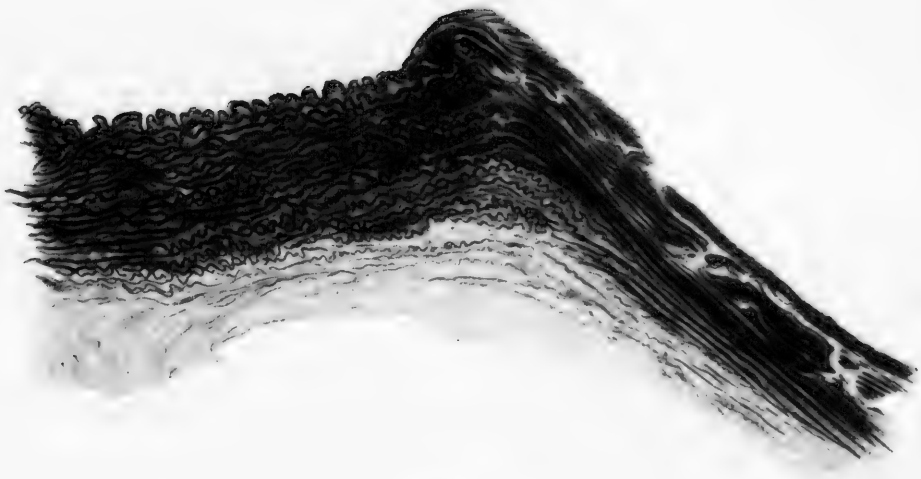

FIG. 4

ON CERTAIN THERMOSTABILE VENOM ACTIVATORS.

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The nature of the cytolytic processes involved in venom cytolysis was made clear through the researches of Flexner and Noguchi, Calmette, and Kyes and Sachs. Flexner and Noguchi [1] first demonstrated that venom contains an intermediary body (amboceptor) for blood corpuscles and, later, they proved that venom also possesses intermediary bodies which are capable of uniting with the somatic cells of a wide series of animals and of bringing them under the solvent influence of extracellular and intracellular complements. The complements which they described are thermolabile bodies; while the researches of Calmette [2] pointed to the existence in serum of a thermostabile body which united with the venom hæmolytic principle and caused hæmolysis, and this body was discovered by Kyes and Sachs [3] to be lecithin. Recently Kyes has produced a crystalline substance which he calls cobra lecithid, which retains the property of bringing about solution of red blood corpuscles.

In order to study further the manner of action of lecithin in respect to its complementing property and to compare it with substances of related nature, Kyes tested other phosphorized fats such as kephalin, protagon, and cerebrin. He found that kephalin[4] was also capable of activating venom, although in less

[1] Flexner and Noguchi, *Journal of Experimental Medicine*, 1902, vi, 277; *Journal of Pathology and Bacteriology*, 1902, viii, 379; *University of Pennsylvania Medical Bulletin*, 1903, xvi, 163.

[2] Calmette, *Compt. rend. Acad. d. Soc.*, Paris, 1902, cxxxiv, 1446.

[3] Kyes, *Berliner klin. Wochenschrift*, 1902, 886, 918; 1903, 956, 982. Kyes and Sachs, *op. cit.*, 1903, 21, 57, 82.

[4] I had also found that kephalin is complementary to venom, but I did not think it worth while to publish the observation after Kyes's paper appeared.

degree than lecithin, while the other two substances were devoid of this power. This fact is of theoretical interest, since kephalin stands closely related to lecithin chemically. On the other hand, Kyes found that cholin, glycerino-phosphoric acid, and some fats were inactive in this respect.

The nature of the case of venom activation calls for a study of the inherent hæmolytic powers of the substances employed as activators, and this should be carried out in a strictly quantitative manner. The great difficulty in the study of the hæmolytic power of lecithin is that this substance is very unstable, and that it seems almost impossible to get a well-defined chemical body. Most of the commercial preparations of lecithin comprise a series of rather different bodies. The impression exists that lecithin is an almost indifferent substance, while as a matter of fact some preparations of lecithin are powerful hæmolyzing agents. The source of misapprehension is to be found, on the one hand, in the variation of the substance called lecithin, and on the other, in the velocity of reaction, which is often overlooked. If we compare the hæmolysis caused by cobra venom plus lecithin with that of lecithin[5] alone the final effect may be quite the same in both cases, but it is obtained much more quickly in the first case. The difference may in some cases be so great that the reaction is finished almost instantaneously with cobra plus lecithin, while it requires six hours or more for its completion with lecithin alone. I can point to other examples of great difference in the velocity of reaction, but not in the degree of hæmolysis. Equivalent amounts of most acids produce the same final hæmolysis, but the rapidity is much greater for the strongly dissociated acids.

Kyes studied the hæmolytic value of lecithin, and he found that, as compared with lecithin in the presence of venom, it was 200 to 300 times less active in the pure state. This estimation must be considered as conditional upon the manner of experi-

[5] Madsen and Noguchi, "Toxines et antitoxines," *L'influence de la temperature sur la vitesse de réaction*, ii; *Académie royale des sciences et des lettres de Danemark; Bulletin de l'année 1904*, 447; and *Centralbl. f. Bakt. und Parasitenk.* 1905, xxxvii, Abt. i., Referate, 356.

mentation. Venom-lecithin hæmolysis is complete within fifteen minutes, while simple lecithin hæmolysis is, as I have pointed out, complete only after about six hours. Hence, an absolute comparison of activity should be made not at once but after the lapse of at least this period. That it is essential to study the end reaction will be conceded when it is considered that the proportion at an early stage between lecithin hæmolysis and venom-lecithin hæmolysis may be as 1 to 1000, while at the end of the process it may be only as 1 to 20. A quantitative study of the reactions has indeed shown me that, as measured by the constant of the end of the reaction, the difference in hæmolytic activity of the pure lecithin as compared with the lecithin and cobra venom mixture is as 1 to 16. This proportion indicates the activating strength of the lecithin used by me; and a determination of the activating strength of other substances may equally be made by bearing in mind and observing the reaction velocity. It is in this way alone that any knowledge of absolute strength can be obtained. The present investigation had for its purpose the study of other substances than those already studied by Kyes with the view of ascertaining whether similar activating properties would be exhibited by any of them.

METHODS.

Materials.—All the chemicals except lecithin were procured directly from Merck at Darmstadt immediately before being used. The lecithin was obtained, first, from hens' eggs, and was prepared by Dr. Madsen at this Institute; and, second, from the cardiac muscle of the ox, this sample having been prepared for me by Professor Bock at the Pharmacological Laboratory of the Copenhagen University.

The solutions were made normal, and employed as such with the few exceptions in which percentage solutions were used. All water-soluble chemicals were dissolved in distilled water, while the insoluble ones were emulsified in water by means of an equal weight of gum arabic. Lecithin, while insoluble in water, can be emulsified readily without the use of the suspending gum. Triolein, tripalmitin, and tristearin are not emulsified

by these means; but, by first dissolving them in a small amount of ether and then shaking them with a larger quantity of methyl alcohol, they can be rendered emulsifiable. At least triolein can be suspended in this way, while tripalmitin and tristearin were never rendered wholly satisfactory for experiment. The solutions of natrium oleinicum, natrium stearinicum, and natrium palmitinicum, on account of the liability to dissociation, were always freshly prepared before being used.

The venoms employed were cobra, water moccasin, habu (Trimeresurus), and daboia. They were dissolved in 0.9 per cent. salt solution.

The blood corpuscles were those of the horse washed twice in salt solution and suspended in the proportion of 1 part of the condensed corpuscles to 99 parts of the 0.9 per cent. salt solution. These washed corpuscles are not hæmolyzed by any of the venoms alone. Hence, they are suitable for such an investigation as the present.

Test of Activation.—Activation of venom can be accomplished by means of two kinds of substances: First, those which have no inherent capacity to cause hæmolysis, and, second, those which, while causing independent hæmolysis, act in weaker concentrations in the presence of venom. In the latter case the velocity of the reaction is, as a rule, and especially with lecithin, accelerated. The thermolabile serum complements belong to the first and lecithin and some other chemical substances to the second class of substances.

Technique.—Each tube contained 8 cubic centimeters of the 1 per cent suspension of condensed horse's corpuscles. To each tube varying amounts of the substance to be tested for activation were added, and, at the same time, in the main series, 0.2 cubic centimeters of a 0.3 per cent. solution of cobra or other venom. The mixtures were first thoroughly shaken, then placed at 37° C. for two hours, and kept fifteen hours longer at 15° C. The reading was made at the end of this time, i. e., 17 hours. In testing suspended, insoluble substances, several shakings were given during this period.

The estimation of the hæmolytic power was made on the basis

of the extremes of action, namely, the quantity which sufficed to produce a trace and the quantity required just to produce complete hæmolysis. Control tubes, minus the activator and venom, were exposed in every series in duplicate exactly to the same conditions of shaking, temperature, etc.

The degree of dilution of the normal solutions was adjusted according to the activity of the substance. The stronger the native activity of the substance the lower the degree of normal strength, and *vice versa*. In this way the quantity of normal solution added was made to vary between 0.2 cubic centimeter and 1 cubic centimeter. In spite of their feeble power as activators, the higher fatty acids could not be used in stronger concentrations than $\frac{1}{10}$ N. on account of their high molecular weights; on the other hand, all the higher acrylic acids and their salts and lecithin[6] displayed such high power that they were employed in concentrations varying from $\frac{1}{100}$ N. to $\frac{1}{1000}$ N.

Table I gives the results with each substance tested, the calculation having been made on the basis of 1 N. solution, unless special mention to the contrary is made. The sign — indicates no hæmolysis with the highest concentration employed.

An examination of this table will reveal certain new facts. Many acids produce hæmolysis, but a striking difference is apparent between the action of the normal fatty acids and the monocarbonic acrylic acids. The hæmolytic power of the normal fatty acids seems to become weaker with the higher members until, with some of the higher acids, no hæmolyzing effect is produced. The reverse is true of the acrylic monocarbonic acids, since with them the larger the number of carbon atoms the stronger is the hæmolytic power exerted by them.

The disappearance of the hæmolytic power in the higher

[6] The molecular weight of lecithin is rather indefinite, but in this case I have taken it for a dioleinic lecithin ($C_{44}H_{86}O_9NP = 807$), and it was made into a 0.807 per cent. emulsion corresponding to $\frac{1}{100}$N. From this concentration any desirable quantity was made by means of addition of a certain quantity of 0.9 per cent. NaCl solution. The figures given in the tables are calculated on the basis of 1 N. in order to make possible the comparison of the results with those obtained with more weakly acting chemicals.

TABLE I.

Name.	Native hæmolytic power.		Hæmolytic power upon the venomized corpuscles.	
	Dose for C.H.	Dose for Trace H.	Dose for C.H.	Dose for C.H.
Acid. formicum	0.018	0.01	0.018	0.01
" aceticum	0.018	0.01	0.018	0.01
" propionicum	0.018	0.01	0.018	0.01
" butyricum	0.018	0.01	0.018	0.01
" palmitinicum	0.05	0.015	0.007	0.002
" stearinicum	—	0.08	—	0.018
" arachinicum	—	—	—	—
" ceratinicum	—	—	—	—
Acid. crotonolicum	0.025	0.015	0.005	0.001
" nonylicum	0.025	0.01	0.01	0.005
" undecylenicum	0.018	0.01	0.006	0.0004
" oleinicum	0.002	0.0008	0.0002	0.00005
" elaidenicum	0.02	0.0025	0.003	0.0002
Acid. ricinolicum	0.015	0.002	0.003	0.00015
Acid. maleinicum	0.013	0.008	0.013	0.008
" fumaricum	0.011	0.008	0.011	0.008
" citraconicum	0.015	0.008	0.015	0.008
" itaconicum	0.017	0.01	0.017	0.01
Acid. sorbinicum	0.017	0.01	0.017	0.01
Acid. oxalicum	0.014	0.006	0.014	0.006
" succinicum	0.012	0.005	0.012	0.005
" tartaricum	0.012	0.005	0.012	0.005
" citricum	0.008	0.0035	0.008	0.0035
Acid. glycerino-phosphoricum	1.	0.05	1.	0.05
Acid. hydrochloricum	0.018	0.01	0.018	0.01
" sulphuricum	0.009	0.006	0.009	0.006
" phosphoricum	0.009	0.007	0.009	0.007
Natrium stearinicum	0.03	0.015	0.015	0.005
" oleinicum	0.0008	0.0005	0.0002	0.00005
" crotonolicum 1 %	0.4	0.1	0.15	0.02
Tripalmitin	—	—	—	—
Tristearin	—	—	—	—
Triolein	0.0018	0.0006	0.00008	0.000015
Lecithin (egg)	0.002	0.00065	0.00012	0.00004
" (ox's heart), unknown concentration	2.	1.2	0.2	0.08
Neurin 1 %	1.5	1.	1.5	1.
Cholin 1 %	1.5	1.	1.5	1.
Alcohol methylenicus 10 N	2.	1.5	2.	1.5
" ethylenicus 10 N	1.6	1.	1.6	1.
" allylicus 10 N	0.5	0.3	0.07	0.03

normal acids may be due in part to their insolubility in water and in part to the reduction of the reaction velocity. That insolubility in water is not the only factor is shown by the behavior of the acrylic acids, which, while also insoluble in water, are, nevertheless, strongly hæmolytic. It is interesting to note that an isomer of oleinic acid, namely elaidenic acid, possesses much weaker action than the former acid, and, further, that ricinolic acid, the oxydate of oleinic acid, exhibits about the same power as elaidenic acid. It may be pointed out in this place that oleinic acid, sodium oleate, triolein, and lecithin possess the strongest hæmolytic action of all the chemicals studied, sodium oleate causing $2\frac{1}{2}$ to 3 times the degree of hæmolysis produced by oleinic acid or lecithin, twice as much as triolein, and $22\frac{1}{2}$ times as much as hydrochloric acid or the lower normal fatty acids.

With tripalmitin and tristearin no hæmolytic action was noted, although they were used in concentrated (1 N.) ethereal solutions, the volume of ethereal solution being, of course, limited to subhæmolytic amounts (to 8 c.c. less than 0.15 c.c.). However, the results with these substances are inconclusive.

The hæmolytic action of cholin and neurin is about the same, and the degree is about that of most monovalent acids. On the other hand, the power of the alcohols is weak, methyl alcohol standing below ethyl, and ethyl below allyl alcohol in hæmolytic power. It may be pointed out that the saturated alcohols tested were weak agents as compared with the far more active unsaturated allyl alcohol.

With this preliminary study of the hæmolytic activity of different chemicals we can proceed to a similar study of the same bodies in the presence of venom. Hitherto, there have been carefully studied, in this respect, only lecithin and kephalin and certain overheated serums in which the activating substances are probably of lecithin-like nature. Kyes, however, noted that a few neutral fats and certain salts of fatty acids activate venom in a minor degree.

Since I have shown that a relation or constant exists between the inherent and the venom activating hæmolytic power of certain chemicals, I felt the need of a standard with which any

of the active chemicals can be compared. For the purpose of this standard, I chose lecithin. According to my own experiments, lecithin possesses the following power: In 1 N. suspension the "complete" hæmolytic dose is 0.002; the "trace" hæmolytic dose, 0.00065. In the presence of cobra venom the "complete" dose is 0.00012, the "trace" dose, 0.00004. Hence, by the addition of cobra venom, lecithin is raised in power for complete hæmolysis according to the equation $\frac{0.002}{0.00012}$, or 16.6 times, and for traces of hæmolysis, $\frac{0.00065}{0.00004}$, or 16.25 times. And since lecithin has been considered to be an activator in the true sense, as it combines with the venom hæmolytic amboceptor to form a compound,[7] we may, for the present, call any substance, which is raised in power by venom in similar degree, a venom activator. Certain chemicals in the following table fulfill this requirement.

While the substances enumerated in Table II would appear to be activating in an unmistakable way, they certainly fulfill the requirements of my definition, although in different degrees. The strongest body in this respect, of those tested, is triolein, and as compared with the weakest body, nonylic acid, it is twenty times as powerful an activator. It is interesting to recall that the three strongest activators—triolein, oleinic acid, and lecithin—are all related chemically to each other. That they do not act in a peculiar manner in the presence of venom and are not alone raised to multiples of their former hæmolytic strength through its presence, is shown by the conduct of such weak activators as ricinolic, undecylenic, crotonolic, and perhaps also stearinic acid, which, while showing comparatively small inherent activity, are raised in about the same proportion when tested in the presence of venom. On the other hand, the body, natrium oleate, which possesses the strongest inherent hæmolytic powers, is one of the poorer activators. In the presence of venom, oleinic acid is made as strong a hæmolyzing agent as natrium oleate; in acting without venom the latter is two and a half times as strong as oleinic acid.

The activity limits, for the production of traces and com-

[7] *Cf.* Kyes, loc. cit.

TABLE II.

	Complete hæmolysis.				Trace of hæmolysis.				Through venomization the hæmolytic power is increased by so many times.	
	Without venom.		With venom.		Without venom.		With venom.			
	Dose in c.c. 1 N. solution.	Hæmolytic units per 1 c.c.	Dose in c.c. 1 N. solution.	Hæmolytic units per 1 c.c.	Dose in c.c. 1 N. solution.	Hæmolytic units per 1 c.c.	Dose in c.c. 1 N. solution.	Hæmolytic units per 1 c.c.	For complete H.	For trace of H.
Lecithin	0.002	500.0	0.00012	8333.3	0.00065	1538.4	0.00004	25000.0	16.6	16.25
Triolein	0 0018	555.5	0.00008	12500.0	0.0006	1666.6	0.000015	66666.6	22.5	40.
Acidum oleinicum	0.002	500.0	0.0002	5000.0	0.0008	1250.0	0.00005	20000.0	10.	16.
" ricinolicum	0.015	66.6	0.003	333.3	0.002	500.0	0.00015	6666.6	5.	13.3
" elaidenicum	0.02	50.0	0.003	333.3	0.0025	400.0	0.0002	5000 0	6.6	12.5
" undecylenicum	0.018	55.5	0.006	166.6	0.01	100.0	0.0004	2500.0	3.	25.
" nonylicum	0.025	40.0	0.01	100.0	0.01	100.0	0.005	200.0	2.5	2.
" crotonolicum	0.025	40.0	0.005	200.0	0.015	66.6	0.001	1000.0	5.	15.
" palmitinicum	0.05	20.0	0.007	142.6	0.015	66.6	0.002	500.0	7.1	7.5
" stearinicum	0.2	5.0	0.08	12.5	0.2	5.0	0.018	55.5	2.5	11 1
Natrium "	0.03	33 3	0.015	66.6	0.015	66.6	0.005	200.0	2.	3.
" crotonolicum	0.04	25 00	0.015	66.6	0.01	100.0	0.002	500.0	2.6	5.
" oleinicum	0.0008	1250.0	0.0002	5000.0	0.0005	2000.0	0 00005	20000.0	4.	10.
Alcohol allylicus	5.0	0.2	0.7	1.4	3.0	0.3	0.3	3.3	1.5	10.

plete hæmolysis, are quite widely separated by venomization, the increase being greater at the lower limits. The reason for this difference has not been established, but it may to some extent be connected with the manner of distribution of the insoluble bodies in the fluid. In the presence of the smaller quantities of insoluble substance a more minute distribution is possibly obtained, thus favoring union with the venom and action upon the corpuscles. What this difference depends on is at present merely speculation; a special study would be required for its determination. The rate of hæmolysis in the presence of venom differs markedly for the different chemicals. With lecithin the reaction is rapidly produced, while with the rest of the activators it proceeds much more slowly. All the other hæmolytic substances tested, whether organic or inorganic, acted indifferently upon the normal and the venomized corpuscles. Possible exceptions are methyl and ethyl alcohols, in which a slight increase of hæmolysis was observed in the presence of venom.

In the course of my work I have used other venoms than cobra poison. I have not tested all the chemicals anew with them, but have confined my studies to lecithin, triolein, and oleinic acid. The manner of carrying out the tests was slightly modified. Twice-washed horse corpuscles (1 per cent in 0.9 per cent NaCl sol.) were mixed with uniform quantities of the three activators mentioned. The quantities employed were insufficient to produce inherent hæmolysis, but sufficient to bring about this change in the presence of venom, which was added in varying amounts. The mixtures were kept at 37° C. for two hours and at 15° C. for fifteen hours subsequently. The blood suspension was 8 cubic centimeters, the activator 0.0002 of a 1 N. solution, and the venom in 1 per cent solution of the dried poison, varying quantities per tube. Table III gives the detailed results.

It will be observed that all the venoms are activated by the three chemicals employed, and, excluding a few irregularities, these range themselves in the following order of activity: triolein, lecithin, oleinic acid. On the other hand, the reaction velocity is greatest with lecithin. One special fact may be men-

TABLE III.

Kind of venom.	Cobra 1 %.		Water Moccasin 1 %.		Trimeresurus. 1 %		Daboia 1 %.		Crotalus 1 %.	
Kind of activator.	Dose for C. H.	Dose for trace H.	Dose for C. H.	Dose for trace H.	Dose for C. H.	Dose for trace H.	Dose for C. H.	Dose for trace H.	Dose for C. H.	Dose for trace H.
Lecithin 1 N. 0.0002.	0.04	0.01	0.06	0.02	0.012	0.004	0.04	0.014	0.2	0.4
Triolein 1 N. 0.0002	0.03	0.01	0.03	0.01	0.006	0.002	0.03	0.01	1.5	0.5
Oleinic Acid 1 N. 0.0002	0.05	0.016	0.05	0.016	0.02	0.01	0.04	0.01	2.5	1.

tioned about Crotalus venom. According to the results of these experiments, it appears to be a poor hæmolyzer, being 50 times weaker than cobra, water moccasin, and daboia venoms, and 100 to 150 times weaker than Trimeresurus venom. If, however, a more appropriate activator is employed, this difference disappears to a large extent. In the presence of dog's serum, whether fresh or after heating, the relative strength rises as compared with Trimeresurus as 1 to 10, and at the same time the reaction velocity is much accelerated. It is very probable that dog's serum, and perhaps other serums, contain thermostabile activators especially adapted to this venom. I might mention that several years ago I isolated from dog's serum, by ether extraction, a grayish hygroscopic powder, which was highly activating for Crotalus venom. I was unable at the time to identify this substance and I have not been able to return to its study.

As regards the quantitative relations of venom and activator, it may be said that the degree of hæmolysis depends on the relative quantities of each substance used. If to a given quantity of blood suspension more venom is added, then less activator is needed, and *vice versa*. Morgenroth and Sachs [8] studied the quantitative relations of serum hæmolysis, and Kyes has made observations on cobra venom and lecithin regarding the degree of hæmolysis, from which he concluded that the greater the quantity of venom employed the less the amount of lecithin needed. No study having been made of the more delicate quantitative relations of thermostabile activators and venom, I have tried, in

[8] Morgenroth and Sachs, *Ehrlich's Gesammelte Arbeiten zur Immunitätsforschung*, 1904, 359.

this paper, to supply that deficiency. I have studied from this point of view cobra venom and three activators, namely, lecithin, triolein, and oleinic acid.

Series A. Venom Uniform, Activator Variable.—Each tube contained 8 cubic centimeters of 1 per cent. horse's washed corpuscles in 0.9 per cent. NaCl. The tubes, after receiving the venom and activators were kept at 37° C. for two hours and at 15° C. for four hours. Table IV gives the details of the experiment.

TABLE IV.

Cobra venom 1 %.		1 N. Lecithin.		1 N. Triolein.		1 N. Oleinic acid.		
Uniform dose for each set of tubes.	Proportion.	Amount for C. H.	Proportion.	Amount for C. H.	Proportion.	Amount for C. H.	Proportion.	Square root of proportion of venom.
i. 0.0025	1	0.0005	1	0.0003	1	0.0009	1	1.
ii. 0.005	2	0.00035	1:1.4	0.00025	1:1.2	0.0006	1:1.5	1.41
iii. 0.01	4	0.00025	1:2.0	0.00016	1:1.9	0.0004	1:2.25	2.
iv. 0.02	8	0.00018	1:2.5	0.00012	1:2.5	0.0003	1:3.0	2.83
v. 0.04	16	0.00012	1:4.1	0.00008	1:3.75	0.0002	1:4.25	4.
vi. 0.08	32	0.0001	1:5.0	0.00005	1:6.0	0.0001215	1:9.0	5.66
vii. 0.16	64	0.000075	1:6.6	0.00004	1:7.5	0.0001	1:9.0	8.

From this table the conclusion can be drawn that an increase in the quantity of venom does not reduce markedly the requirement in activator, although it reduces the requirements systematically. The definiteness of the relation between venom and activator is striking in spite of a few irregularities, for which technical errors may easily account, and it may be expressed in the form of a rule which might be framed as follows: *The requirement in activator for producing an equal degree of hæmolysis of horse's corpuscles is in proportion to the square root of the amount of venom present in the mixture.*

Series B. Activator Uniform, Venom Variable.—This experiment is the reverse of the preceding. All conditions were the same as in Series A, except that with a constant amount of activator, varying quantities of venom were employed. The results are given in Table V.

A study of this table suffices to show that the degree of action of the activator is different from that of the intermediary body, and that as the former is increased the latter may be diminished

TABLE V.

1. LECITHIN + COBRA VENOM.

1 N. Lecithin.			Cobra venom 1 %.		Square of proportion of the amount of lecithin.
	Uniform dose for each set.	Proportion.	Dose for C.H.	Proportion.	
i.	0.00005	1	0.3	1	1
ii.	0.0001	2	0.08	1: 3.8	4
iii.	0.0002	4	0.02	1: 15.0	16
iv.	0.0004	8	0.005	1: 60.0	64
v.	0.0008	16	0.0015	1: 200.0	256

2. TRIOLEIN + COBRA VENOM.

1 N. Triolein			Cobra Venom 1 %.		Square of proportion of the amount of triolein.
	Uniform dose for each set.	Proportion.	Dose for C.H.	Proportion.	
i.	0.00005	1	0.275	1	1
ii.	0.0001	2	0.08	1: 3.4	4
iii.	0.0002	4	0.025	1: 11.0	16
iv.	0.0004	8	0.0045	1: 55.5	64
v.	0.0008	16	0.0012	1: 230.0	256

3. OLEINIC ACID + COBRA VENOM.

1 N. Oleinic Acid.			Cobra Venom 1 %.		Square of proportion of the amount of oleinic acid.
	Uniform dose for each set.	Proportion.	Dose for C.H.	Proportion.	
i.	0.0001	1	0.225	1	1
ii.	0.0002	2	0.06	1: 3.8	4
iii.	0.0004	4	0.0125	1.18.0	16
iv.	0.0008	8	0.003	1: 75.0	64

without failure to cause hæmolysis. In this instance a rule may also be formulated, which might read: *An increase in activator permits of a reduction in venom approximately in proportion of the square of the amount of activator employed.*

Inhibition of Lecithin-Venom Hæmolysis by Cholesterin.—While we[9] failed to observe definite protective action of cholesterin against venom-complement hæmolysis, Kyes[10] found that cholesterin is capable of preventing venom-lecithin hæmolysis. I have experienced no difficulty in confirming this observation of Kyes, and I have extended my study of the subject to the question as to whether cholesterin acts upon the venom hæmolysin, or upon the activator.

The manner of study was as follows: A series of tubes was prepared, containing venom in increasing and lecithin in decreasing amounts. According to this plan and in keeping with the observations made in an earlier part of this paper, several different hæmolytic mixtures will be obtained. To these mixtures cholesterin is added in exactly the quantity needed to prevent hæmolysis. If the amount of cholesterin required is proportional to the venom used, the presumption would naturally be that it reacted with that substance, while if it is proportional to the lecithin, then the presumption would be in favor of reaction with that body. Should the amount of cholesterin remain about constant throughout the experiments, the probability would be that it acted upon the blood corpuscles and protected them directly from hæmolysis.

Five series of tests were made. The orders were as follows: i. Venom + lecithin + cholesterin + blood; ii. venom + cholesterin + lecithin + blood; iii. lecithin + cholesterin + venom + blood; iv. blood + lecithin + cholesterin + venom; v. blood + venom + cholesterin + lecithin. The results of the tests are given in Table VI which follows.

Table VI shows that a relation exists between the cholesterin and lecithin in the mixtures and not between cholesterin and venom or corpuscles, and this fact seems to prove conclusively that the antihæmolytic action of cholesterin depends on its reaction with lecithin. In order that lecithin-venom hæmolysis may be prevented by cholesterin, a certain interval of action between the lecithin and cholesterin is required with minimal

[9] Flexner and Noguchi, loc. cit.

[10] Kyes, loc. cit.

TABLE VI.

	Combination of venom, lecithin, cholesterin,* and blood suspension.	Cobra venom 0.2 % plus lecithin 1 N.	Correlative amounts of venom and lecithin, representing units of hæmolytic power.			
			0.1 0.0004	0.2 0.0003	0.4 0.0002	1.6 0.0001
i.	Venom + lecithin, 6 hours—36° C. + *cholesterin*, 15 minutes—15° C. + blood. " + " " " " " 3 " " —15° C. + " " + " 3 min. —36° C. + " " " + "	Takes 1 N. cholesterin. " " " " " "	0.0008 0.0012 0.0012	0.0006 0.0010 0.0010	0.0004 0.0007 0.0008	0.00016 0.00036 0.0004
ii.	Venom + *cholesterin*, 6 hours—36° C. + lecithin, 15 minutes—15° C. + blood. " + " " " " " 5 " " + " " + " 3 min. — 36° C. + " " " + "	" " " " " " " " "	0.0008 0.0012 0.0012	0.0006 0.0010 0.0010	0.0004 0.0008 0.0007	0.00016 0.00032 0.00036
iii.	Lecithin + *cholesterin*, 6 hours—36° C. + venom, 15 minutes—15° C. + blood. " + " " " " " 3 " " + " " + " 3 min. —36° C. + " " " + "	" " " " " " " " "	0.00076 0.0008 0.0012	0.00058 0.0006 0.0010	0.00036 0.00036 0.0006	0.00016 0.00016 0.00036
iv.	Blood + lecithin, 60 min.—36° C. + *cholesterin*, 15 minutes—15° C. + venom.	" " "	0.0008	0.0006	0.0004	0.0002
v.	Blood + venom, 30 min.—36° C. + *cholesterin*, 15 minutes—15° C. + lecithin.	" " "	0.0018	0.0012	0.0008	0.0004

* The amount of cholesterin used was contained in 0.2 c.c. of methyl alcohol.

quantities of cholesterin, while with larger amounts of this substance the time interval is greatly diminished.

It is probable that the reaction between cholesterin and lecithin depends on their solubilities in each other, and, hence, if the conditions are so modified as to alter essentially their solubilities, the result may be quite or wholly changed. That this is true can be shown by the action of methyl alcohol, which is capable of setting aside the antihæmolytic action of cholesterin. Thus, if 0.4 cubic centimeter of methyl alcohol is added to 8 cubic centimeters of the mixture of horses' corpuscles, venom, and lecithin, the quantity of cholesterin required to prevent hæmolysis will be greatly increased, while a quantity of the alcohol over 0.8 cubic centimeter so changes the conditions as to render cholesterin impotent in preventing, although it may still delay, hæmolysis as Table VII exhibits.

TABLE VII.

Hæmolytic mixture. Amount of methyl alcohol.	Cobra venom 0.2 % Lecithin 1 N.	0.1 0.0004.	0.2. 0.0003.	0.4. 0.0002.	1.6. 0.0001.
0.1	Cholesterin 1 N.	0.0007	0.00056	0.0004	0.00016
0.2	" "	0.0008	0.0006	0.0004	0.00018
0.3	" "	0.0010	0.0008	0.0005	0.00022
0.4	" "	0.0025	0.0020	0.0015	0.0008
0.5	" "	0.0060	0.0040	0.0028	0.0015
0.6	" "	0.0100	0.0080	0.0050	0.0040
0.7	" "	0.0400	0.0300	0.0200	0.0150
0.8	" "	Hæmolysis retarded, but no protection.			
1.0	" "	"	"	" "	"

This study was carried out while I was being maintained abroad by a grant from the Carnegie Institution of Washington, D.C., and it is a continuation of the studies on venom which I began at the University of Pennsylvania with Professor Flexner, in which I had the support and advice of Dr. S. Weir Mitchell.

I desire to thank Dr. Madsen, the director of the Statens Serum Institute, for many acts of kindness during my stay in his laboratory.

A FURTHER CONTRIBUTION TO THE KNOWLEDGE OF MULTIPLE NON-INFLAMMATORY NECROSIS OF THE LIVER WITH JAUNDICE (HEPAR NECROTICUM CUM ICTERO), AND TO THE KNOWLEDGE OF CELL DEGENERATION AND CYTOLYSIS IN GENERAL.[1]

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PLATE VIII.

In a previous communication [2] I described under this title the anatomical and histological findings of a peculiar affection of the liver, which could not be grouped under any of the headings now in general use in the classification of the diseases of that organ, and which presented interesting general pathological features in the form of a peculiar cellular destruction, unassociated with any inflammatory reaction and unlike that usually observed in the disintegration of parenchymatous cells.

To recapitulate, the process consisted of a multiple, irregular, circumscribed solution of liver cells, without parenchymatous degeneration or coagulation necrosis, and associated with a corresponding lobular blood and bile stasis in the affected areas. It appeared that the protoplasm and later the nucleus of the cells simply wasted and dissolved, leaving a more or less well-preserved reticulum with stagnant bile and blood. While undergoing this change the cells showed fatty degeneration, and finer or coarser bile pigment within their bodies, sometimes reaching a considerable degree, conditions to which I shall refer in detail later. The impression one gained from the appearance was that of a solution or cytolysis of the parenchyma. The process was as a rule more marked in the central portions of the lobules, taking its origin around the central vein, but by no means always confined to that point, as it occurred also in streaks running from one into neighboring lobules, and gradually was lost in well-preserved liver tissue. Moderate peripheral fatty infiltration existed, but there was an entire absence of inflammatory reaction within the liver tissue

[1] This study was aided by an appropriation from the Rockefeller Institute for Medical Research.

[2] *Journal of Medical Research*, 1904, xii, 75.

itself, the portal spaces alone showing sclerosis of a more or less recent date. The last condition was most pronounced and mature around the portal vein itself.

The whole lesion presented itself as a diffuse affection of the liver, and had not led to any marked gross diminution in size, but the organ had become tough, leathery, pale yellow, bile stained, and the markings had been lost. Irregular vascular injection or darker, larger, deep red spots (corresponding to the irregular stasis) were plainly visible.

Two features then made this observation of particular interest. First, the peculiar destruction of liver cells which did not correspond to the usual atrophy, degeneration, or necrosis of parenchymatous cells, second, the entire lack of acute or chronic inflammatory reaction of the liver tissue, for the affected areas showed only the passive feature of the cellular waste. Further, the well-confined sclerosis of the portal spaces was more mature around the vessels than around the proliferating bile ducts.

I explained in my first communication that we had here a condition that could not be grouped within the present classification of the diseases of the liver, and on account of the general severe destruction of liver tissue and the generalized jaundice I suggested the term of multiple non-inflammatory necrosis of the liver with jaundice (Hepar necroticum cum ictero).

In looking through the literature for similar or related cases I was able only to find a number of observations by Curschmann,[3] under the title of a peculiar form of necrotic hepatitis, under which name is described, as following long-continued obstructive jaundice, somewhat similar multiple non-inflammatory destruction of liver tissue, but leading to actual softening and breaking down of circumscribed areas. Here also the peculiar feature of a simple loss of liver substance with bile imbibition and an entire lack of inflammatory reaction in or around the affected areas attracted his attention, as well as a well circumscribed but confined sclerosis of the bile ducts of the portal spaces.

Curschmann, however, could not give any evidence as to how the cellular destruction took place in his cases, for they were all far advanced beyond the stage of studying the histogenesis of the lesion. But in the explanation of his cases Curschmann expresses an idea which I utilized in the explanation of my case. It was based largely on the clinical history, but also on some of the additional anatomical and histological findings. He believes that his cases cannot be understood on the basis of bile stagnation alone, but that it is necessary to assume an additional infection, and recalls that certain bacteria, like the bacterium coli introduced into the bile channels, do not necessarily produce suppuration, but a simple general infection. In the case that I had observed we

[3] "Ueber eine eigenartige Form der nekrotisirenden Hepatitis," *Deutsches Archiv. für klinischen Medizin*, 1899, lxiv, 564.

had to do with an individual addicted to chronic alcoholism who, although a young man, showed mental and anatomical alterations incident to such abuse; and I assumed that in such cases a portal infection acting in a peculiar manner might easily occur and reach the liver, which as a consequence of constant exposure to harm was of low resistance and reacting ability. In favor of this portal entrance of an irritant was the marked localized sclerosis around the portal vessels, analogous to the thickening of the bile ducts in Curschmann's cases.

I advanced at that time no positive opinion as to the exact pathogenesis of the lesion, but it was natural to suppose that it was of bacterial origin, and the peculiar cytolytic changes as well as the entire absence of inflammatory reaction were somewhat more in favor of the action of a bacterial product than of a bacterium itself. However, in the absence of more data I did not enter into detailed discussion of the facts, the apparent rarity and peculiarity of the process inducing me to place it on record as an individual case.

Since that time, that is, for a period of a year and a half, I had particular attention paid in this institute to the various affections of the liver, and we have been able to collect three more cases that belong to the same category, which I now wish to add to my first contribution to the subject, as enlarging upon this peculiar and little appreciated affection. For reference and sake of convenience I may be allowed to quote and somewhat enlarge upon the findings of the first published case.[4]

Case I.—M. G., Irish, longshoreman, thirty years old, was admitted to the City Hospital, Oct. 8, 1903. He had been drinking heavily since the beginning of July, and was in an extremely nervous and mentally somewhat confused condition. His appetite was good, but he vomited some, had night sweats, and a slight cough. His previous history was that he had always been a very heavy drinker, and that he had been confined in bed on several occasions because of alcoholism. The only other disease which he admits is bronchitis. Both parents and one brother died of tuberculosis.

Physical Examination.—Patient jaundiced, but of good nutrition and development. Lungs reveal nothing of importance. Heart: enlarged to percussion from right sternal line to a point slightly beyond the apex beat; systolic murmur at apex. Liver and spleen not felt. Extremities; pretibial pitting. Temperature, 98°. Pulse, 100. Respiration, 24. Urine, reddish yellow, small amount, 1.025, no albumin, no sugar, no casts. During the next two days there was no material change. Temperature varied from 99° to 98°. Pulse, 100 to 104. Respiration, 24. Mind not improved. Jaundice somewhat more marked.

On the third day the patient became violently delirious, and was difficult to

[4] In these histories details apparently not bearing on the question under consideration have been omitted.

restrain, behaving like a case of delirium tremens. His temperature went up to 100°; and on the fourth day to 104°. Pulse, 120. Respiration, 32. He died of marked œdema of the lungs, on October 13, 5 A.M., having been in the hospital not quite five days.

Autopsy Protocol (abbreviated).
(Oertel) Oct. 13th, 2 P.M.

Male, thirty years old, one meter seventy-eight centimeters tall, of good frame and muscular development, and good nutrition. Skin of the whole body and conjunctivæ distinctly jaundiced. No œdema. Rigor mortis marked. Moderate amount of subcutaneous fat, muscle tissue dry and brown. Peritoneal cavity empty. Pericardium almost empty. Right heart collapsed, with dark fluid blood, left heart moderately contracted and empty. Pulmonary and tricuspid valves intact, aortic and mitral valves thickened at base. Heart muscle brownish red, moderately firm. Weight, 555 grams. Left pleural cavity without fluid or adhesions. Left lung moderately emphysematous, firm; on section, deep red, and very œdematous. Right pleura with dense, old, firm adhesions throughout. Lung markedly emphysematous and œdematous.

Spleen.—Weight, 195 grams. Capsule thickened, firm. Section deep red, connective tissue prominent, lymphoid nodules indistinct. *Kidneys* (left).—Slightly enlarged, firm, capsule thickened, moderately but uniformly adherent, surface mottled, markings distinct. Cortex irregular. (Right).—Same. *Adrenals* unchanged. *Stomach.*—Large, dilated with gas, but empty, except for much slimy material. Mucous membrane thickened, much congested. *Intestines.*—Like stomach. Portal structures free.

Liver.—Somewhat larger than normal, weight, 1770 grams; color, dark yellow throughout. Surface on general view smooth, but on closer inspection slightly irregular; consistency firm, leathery. On section cuts firm, vessels distinct and deep red in many places, in others not prominent. Lobules pale yellowish, in spots brownish; their outlines indistinct and irregular. In some places the liver presents a diffuse, yellowish pale appearance, but always firm, nowhere any softening. No connective-tissue bands visible. The gall bladder contains a fair amount of thick, fluid bile; no stones; the larger bile ducts permeable, not dilated, and with no apparent change. The vessels at entrance into liver extensively thickened.

Other organs without important alterations.

Microscopic examination of the liver reveals a most unusual picture. The stained sections show even to the naked eye small irregular streaks of pale, poorly stained areas. On examination these areas (Fig. 1) are found to correspond to wasted portions of liver lobules, usually with a free precipitation of bile in the form of large and small light yellow to dark brown granules. The process leading to loss of the cells is a simple one, without coagulation or extensive parenchymatous or fatty degeneration,

being evidently more like a solution of the protoplasm, preceded by moderate parenchymatous swelling. In my first report I spoke of it as an atrophy. But it differs from ordinary atrophy in a much more rapid, uniform loss of the cellular protoplasm, while the outline of the cell remains visible to the last. One gains the impression that in the affected areas the cellular protoplasm is washed away. The nucleus becomes poorer in chromatin and atrophic, the cells become vacuolated, some contain fat drops, and the cell body then usually encloses smaller or larger granules of bile. Ultimately the cell disappears and only a fine reticulum remains with free bile pigment. The condition is most pronounced around the central vein, which appears well dilated. Towards the periphery of the lobule the cells have retained their integrity, but most of them show beginning turbidity and swelling of the protoplasm, with moderate fatty infiltration. They are also frequently seen to contain fine or coarse granular bile pigment. Their nucleus is poor in chromatin. Such areas are present in immense numbers in various portions of the liver, varying in size and shape, and are directly continuous with apparently normal liver tissue. They are not surrounded by an area of pus or any other infiltration. There is no line of demarcation anywhere, no inflammatory engorgement of vessels, no connective-tissue growth, old or recent, between or within the lobules—in fact, no evidence of any reaction of the liver tissue at all. There is present, however, a pronounced stasis in many places. But this bears no relation to the degree of atrophy or the precipitation of bile, as one may be more pronounced when the other is less conspicuous. In some places, where the waste of the whole central part of the lobule is pronounced, the stagnant blood occupies the space, either free or mixed with bile pigment. The stasis is irregular, not general, not uniformly distributed throughout the whole liver, corresponding, then, to the gross appearance.

Attention is attracted by a characteristic change of the portal spaces, confined to them. The portal vein is generally widely dilated, its walls well thickened, and its neighborhood densely infiltrated with small, round, and connective-tissue cells.

The same is true of the bile ducts and the hepatic vessels. The connective-tissue formation around these structures is frequently more recent and more cellular, than immediately around the portal vein. The sclerosis is confined entirely to the structures of the portal spaces, at no time is there an attempt to surround or invade the lobules. It appears independent of the lesions of the lobules. In some places proliferation of bile ducts, which may reach a considerable degree, is going on. The new ducts are well formed, their epithelium intact, and not congested with bile.

Case II.—D. B., Irish, shoemaker, 48 years old, was admitted to the City Hospital, October 7, 1904, complaining of shortness of breath. He first noticed this disturbance about four weeks ago, when he coughed up loose phlegm; later his cough became tighter, and he could hardly expectorate at all. At irregular intervals he also suffered from stabbing pains in the chest, preventing him from drawing his breath. His family history is negative. The patient has had the ordinary diseases of childhood; eighteen years ago suffered from asthma and bronchitis and since that time has had similar attacks every fall and spring. He admits gonorrhœa many years ago, but denies syphilis. Had been a hard whisky and beer drinker, and smoker, all his life.

Physical Examination.—Patient lies in bed quite dyspnœic, with face and hands deeply cyanosed and a slight unproductive cough. At intervals he is mildly delirious. Chest shows signs of wide-spread emphysema, particularly at the bases. Heart dullness almost entirely obscured by hyper-resonance and the sounds distant. Rhythm irregular, the second sound at the apex reduplicated. Liver enlarged downward to level of umbilicus; tender. Spleen not palpated, and knee jerks cannot be elicited.

Urine: amber, cloudy, acid. Sp. gr., 1.030. Trace of albumin, no sugar, no casts. Temperature, 99° F. Pulse, 128. Respiration, 54. On the next day the patient was bled, about 300 c.c. blood being removed, and his chest cupped; his bowels were regulated, and strychnine administered. Under this treatment he improved somewhat, but soon relapsed, his temperature rose to 101° and 102°, the delirium became more marked, and he died five days after admission, October 12, 1.30 P.M.

Autopsy Protocol (abbreviated).
(Symmers) Oct. 12th, 3 P.M.

Body about 172 cm. long, good frame and muscular development and good nutrition. Skin pale and in parts slightly yellowish, conjunctivæ with slight yellow tinge, no external œdema. On section subcutaneous fat well preserved, muscle tissue dry and pale brown. Abdominal cavity without fluid; small intestines in lower part of belly, collapsed, smooth. Liver visible in median line and right hypochondrium where it extends 5 cm. below the free border of

the ribs, 10 cm. below the sternum in the median line. Diaphragm 4th interspace on right, and 5th on the left side. 100 c.c. fluid in the right pleural cavity, left pleural cavity obliterated. Pericardium contains 25 c.c. of fluid, but free.

Brain.—General marked atrophy of gray matter and thrombotic endarteritis. *Heart.*—With marked dilatation and hypertrophy of the right ventricle, muscle pale and cloudy. *Lungs.*—Marked general emphysema, chronic bronchitis, and irregular œdema. *Spleen.*—Small, atrophic, pulp very dark and prominent, *Kidneys.*—Large, juicy, dark. Cortex with distinct dark glomeruli; swollen, medulla œdematous. *Stomach.*—With well preserved rugæ, and thick, swollen mucous membrane, covered with mucus, and reddened near pylorus. *Pancreas.*—Large, narrow, firm, and pale. On section, pinkish.

Liver. —Somewhat smaller than normal and with localized thickening of capsule. Mottled, partly reddish-blue and partly yellowish-pink surface, but smooth, firm, and leathery. On section it shows towards the periphery of the organ a margin of spotted to more streaky dark vascular injection, but no well defined markings. Towards the centre and including the greater part all markings are lost, and the tissue appears of a diffuse pale yellow color with pointlike, numerous deeper yellow dots, irregularly distributed throughout the whole of the liver substance. The organ is of peculiar firm leathery consistency, but shows nowhere any softening.

Microscopic sections of the liver show to the naked eye an irregular streaky appearance due to an interchange of better and poor stained, pale areas. The pale portions present pinhead to more elongated shapes, and appear well circumscribed. On microscopic examination they correspond to lost or disintegrating groups of liver cells of a peculiar nature (Fig. 2): The protoplasm of the cell loses its staining quality, assumes a hazy, distant appearance, and usually presents moderate irregular swelling; nucleus and protoplasm become then quite indistinct, fade away, larger and smaller fat drops may appear within the protoplasm, the cellular substance seems to dissolve and disappear, but during the whole process the cellular outline is preserved and distinct to the last. Where no fatty change appears the nucleus remains central, where large fat drops occur it is pushed to one side. Ultimately only "ghosts" of cells remain, or a faint, slight reticulum, or even open free spaces which may contain fat, blood, and bile arise. However, blood and bile stasis is here much less pronounced and prominent than in Case I, the wasting cells and subsequent necrotic portions showing a more moderate bile imbibition.

The distribution of the lesion is general, diffuse, but irregular, throughout the whole of the liver. Although frequently taking its origin around the central vein where it appears prominent, it is not so well confined to the central portions of the lobules as in the first case. It appears more irregular and streaky, but the same gradual change to well preserved liver tissue exists. The disintegrated areas show few free small spindle and round nuclei, some leucocytes and nuclear remnants. Nowhere is there evidence of any inflammatory reaction. The portal spaces are here only moderately thickened, the vessels large, and the sclerosis is more marked around the bile ducts. A limited portal bile duct formation coexists. The uninvolved parts of the liver tissue show in part a moderate simple atrophy, but with well preserved and well stained cells, in part slight cloudy swelling, and some fine round and more elongated nuclei may be observed within the lobules. Similar changes are not found in other organs, nor are features present elsewhere that could be brought into connection with the peculiar destruction of the liver.

Case III.—M. E., colored, 28 years old, was admitted to the City Hospital as a transfer case from Harlem Hospital on October 8, 1904. She had been operated upon at that Hospital for what was diagnosed as cirrhosis of the liver, a ventrofixation of the omentum having been performed. Patient was well up to the latter part of August, when she began to feel ill, with loss of appetite and flesh. She states that she had several hæmorrhages from the lungs, and was taken care of in another hospital and improved. Soon after going home her abdomen began to swell, she became very short of breath, and vomited frequently. On going to Harlem Hospital she was operated upon, but did not regain strength, and was therefore transferred to the City Hospital. She complains now of weakness and very poor appetite. Her previous history shows that she has always performed hard domestic work, and that her childhood had been healthy, but she never was very strong; the menses started at 13 years and were always regular. She married at 18, has two children, and had two miscarriages about the seventh month. Family history negative. No alcoholic or syphilitic history.

Physical Examination.—General nutrition poor, skin dry and scaly, with moderate pretibial œdema. Cheeks puffy, lips cyanotic, and external jugular veins greatly engorged. Heart enlarged, extends to outside the nipple line and to right of sternum. A loud blowing systolic murmur at the apex transmitted to the axilla, aortic second sound accentuated. Pulse fast and irregular. Chest emphysematous. Liver felt below costal margin, spleen not palpable. Urine, light yellow, sp. gr., 1.008. Albumin, trace; few hyaline casts. The patient became rapidly worse, her œdema became more pronounced and general, and

the abdomen began to fill; breathing was more and more labored and the heart grew very feeble. A mild delirium prevailed at times. The temperature varied, generally about 99° F. She died on October 28, 6.30 A.M.

Autopsy Protocol (abbreviated).
(Symmers) Oct. 28th, 2 P.M.

Body 145 cm. in length, of delicate frame and poor nutrition. Veins of neck prominent. Old laparotomy scar in right hypochondrium extending vertically downward to 4 cm. below the umbilicus. No marked external œdema; abdomen very prominent.

On section a moderate amount of subcutaneous fat, muscle tissue pale, moist. Abdomen contains a great deal of straw-colored clear fluid; omentum firmly and extensively attached to parietal part of belly wall corresponding to the scar. The peritoneum is generally thickened. Liver presents in epigastrium and right hypochondrium, 8 cm. below free border of the ribs and 12 cm. below sternum. Diaphragm 4th interspace on the right side, and 5th on the left side. Both pleuræ and the pericardium contain considerable clear serous fluid.

Heart.—Hypertrophy of the left ventricle, chronic endocarditis of the mitral valve and cloudy, brown muscle. *Lungs.*—Interstitial pneumonia, emphysema, hydrothorax. *Spleen.*—Very small, pulp prominent dark red. *Kidneys.*—Small, irregularly lobulated, capsule adherent, surface granular, cortex irregular, narrow, markings largely obliterated, fibrous tissue prominent. *Brain.*—Pale, cortex thin. *Stomach and Intestines.*—Show chronic hæmorrhagic inflammation.

Liver.—Large, firm; weight, 1080 grams; covered by partly atrophic and partly thickened capsule; marked variation in color, in places pinkish, in others yellow. On section structure for the most part invisible, consistency leathery. Numerous dark yellow pinhead-size dots distributed throughout a pale diffuse, yellowish-white tissue. Near the surface the organ shows streaky, vascular injection, or more roundish distinctly hæmorrhagic areas.

Here again the microscopic sections of the liver show to the naked eye the same irregular or more elongated streaks of pale non-staining areas, interchanging with well preserved tissue, but the very marked and extensive bile imbibition of the affected areas is at once visible either in a more diffuse yellow tinge of the pale spots or as distinct brownish dots and streaks.

The microscopic examination of these sections shows that we have to deal with an exaggerated process of exactly the same type as the former. Here the cells have mostly disappeared, only shadows of them may occasionally be seen; large fat drops occupy the spaces; the remaining reticulum, however, can be made out more frequently. The capillary blood and bile stasis is irregular but pronounced in all affected areas, the latter es-

pecially being extreme. The wasting cells are filled with, for the most part, coarse dark yellowish-brown pigment and fat drops, and the disintegrated portions contain bile in large clumps, usually mixed with still well preserved red blood cells. The distribution is general and of equal severity throughout the liver. The localization, although the process frequently can be seen to arise around the central vein, is not confined to a particular area of the liver lobule, and the change is well circumscribed. Free nuclei are common here as in the well preserved portions of liver tissue. This latter shows well marked fatty infiltration and moderate patchy parenchymatous swelling. The portal spaces, much as in Case II, show cellular sclerosis about equally prominent around vessels and bile ducts, and a limited, well confined bile-duct formation. There exists no demarcating inflammatory reaction anywhere within the liver tissue, and no line of demarcation between involved and preserved parts.

Case IV.—L. R., colored, male, 65 years old, admitted to City Hospital, December 22, 1904, complaining of asthma and general weakness. He dates this latter condition to a fall from a ladder six or seven years ago, but has suffered from asthmatic attacks more or less all his life. He is a laborer, moderate drinker, and only admits malaria forty years ago. In 1893 he lost his right arm in an accident.

Physical Examination.—Patient's breathing is labored, he has difficulty in speaking and a hoarse rattle in his throat. Entire chest emphysematous and barrel-shaped; jugular veins dilated. Rattle of mucus in bronchi, and loud mucous râles everywhere. Abdomen distended, smooth, tense, recti very rigid. Liver border reaches down about 6 or 8 centimeters below the costal margins and in the median line about the same distance, blending with a small area of cardiac dullness. The heart almost covered by emphysematous lungs. Both legs much swollen. Urine, sp. gr., 1.016, very large amount of albumin, no sugar, many hyaline and granular casts, dark amber color.

Clinical diagnosis: Chronic bronchitis, emphysema, chronic myocarditis, and chronic nephritis.

Subsequent history: The patient's condition practically unchanged but for slight remissions. His temperature was usually about 99° F., once or twice rising to 102°. Sensorium dull. He died on February 6, 1905, 12.05 noon.

Autopsy Protocol (abbreviated).
(Crowell) Feb. 7th, 11 A.M.

Body 168 cm. long, of poor nutrition and musculature, no external œdema except about the ankles. On section small amount of pale œdematous fat,

muscle tissue pale and moist. On opening abdomen large omentum found pulled up over stomach to left hypochondrium, intestine dilated. Liver 4 cm. below free border of ribs on right side and 9 cm. below xyphoid. Gall bladder visible, interlobar septum in median line. Diaphragm stands at the 5th rib on either side. On opening thorax lungs retracted, right pleural cavity contains a liter of turbid yellow fluid, left pleural cavity contains ½ liter. Precordial area large, prominent in left lower half of thorax. Pericardium contains 75 c.c. clear fluid.

Heart.—Large, right side engorged, left collapsed, apex formed by left ventricle outside the nipple line. Valves with moderate thickening. Left ventricle large; at apex, involving an area of about 4 to 5 cm. in length, beginning within the muscle and extending to the surface there is a recent anæmic infarct, surrounded by hæmorrhagic infiltration. The rest of heart muscle pale, cloudy, very fibrous; thickness at mitral, 1.5 cm. Larger coronary vessels not obstructed. Wt., 680 grm. *Left Lung.*—Emphysematous and very œdematous, bronchi with much purulent exudate, diffusely reddened. Vessels free. *Right Lung.*—Upper and middle lobes with large, recent hæmorrhagic infarcts. Lower lobe emphysematous and œdematous. *Spleen.*—Small, with scar of an old infarct, generally atrophic; interstitial tissue marked. *Suprarenals* (both).—Atrophic, moderate amount of pigment. *Left Kidney.*—With little perinephric fat. Capsule thin, strips with ease except for localized adhesions. Surface deep red. Cortex moderately contracted, glomeruli prominent; hyalin. Pyramids deep red, indistinct markings. Vessels prominent. *Right Kidney.*—Similar, but with better preserved cortex. Portal structures free. *Liver.*—Rather small, soft, pale, well marked central veins and prominent interlobular spaces, but pale lobules. Gall bladder with four soft yellow stones. *Stomach and Intestines.*—With atrophic, pale mucous membrane. *Brain.*—Moderate œdema, flattened convolutions; thin, pale cortex.

Microscopic sections of the liver show well circumscribed, limited and streaky solution of the liver cells, as described above, leaving a hazy reticulum, with occasional blood stasis, and general fine and coarse bile imbibition of disintegrating liver cells, and accumulation of small fat drops within the dissolving protoplasm. The process follows more frequently the peripheral parts of the lobules, but occurs also irregularly and in streaks around the central veins. The change to normal liver cells is quite abrupt, and there exists slight parenchymatous swelling and fatty infiltration of the uninvolved areas, but never pronounced bile imbibition or blood stasis. The central veins appear unchanged; the portal spaces show moderate fibrous and sometimes hyalin sclerosis around the vessels; a more cellular growth exists around the bile ducts.

These four cases present a change of the same quality, but

with some quantitative differences. Common to all is the peculiar and characteristic multiple and irregular destruction of the liver tissue, with more or less pronounced bile and blood stasis and fatty change, leading grossly to entire loss of markings,

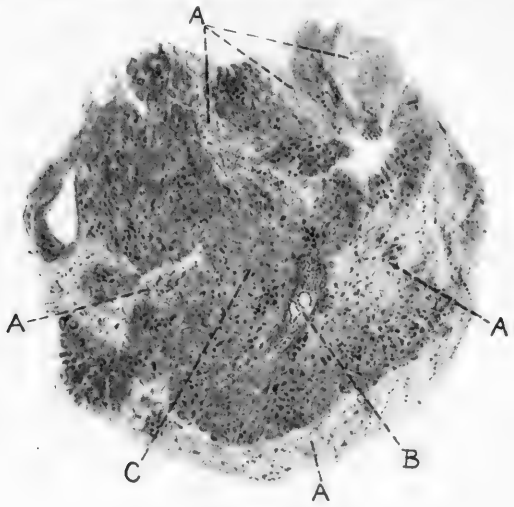

FIG. 1.

leathery consistency, pale yellow color with circumscribed deep yellow or hæmorrhagic spots and streaks, and an entire lack of inflammatory reaction within the liver tissue, and confined portal sclerosis.

It seems certain, as I emphasized in my first report, that we have here a process that differs essentially from the ordinarily observed destruction of parenchymatous cells.[5] It cannot be grouped with the parenchymatous degeneration or necrosis in Virchow's and Weigert's sense, for it does not present the swelling with granular disintegration and coagulation of the protoplasm, with the early loss of well defined cellular outline, and the ultimate production of a structureless mass of dead protoplasm; but, on the contrary, the cell contents show a uniform fading, with the outline well preserved and no particular process, such

[5] There can, in my opinion, be no question of a post-mortem change.

as coagulation or granular disintegration, leading to the death of the cell, only a fatty change frequently being associated with the loss of substance, the ability to secrete bile being retained to a late stage.

The change appears too severe, irregular, and circumscribed to belong to the class of simple atrophies, due to quantitative interference with the nutrition of the cells, nor does its morphology correspond to the latter, for, while in atrophy the protoplasm undergoes a gradual loss from periphery to center, so that some parts of the cell at least are preserved, the general fading is here characteristic. The differences between this and other cellular destructions are evident at a glance. No better term could be given to the lesion than solution of cells, cytolysis, and this lesion assumes importance in view of the recent general interest in the question of cytolysis, as also in relation to some of the experimental results. I shall not attempt to discuss this subject fully in this connection. A rich literature has accumulated during the last few years; I intend therefore only to emphasize certain points that seem to touch upon this morphological observation.[6]

The discovery of autolysis which at first was thought to be entirely of a post-mortem nature was soon to assume importance in the explanation of vital processes. Jacoby was able to demonstrate autolysis by finding leucin and tyrosin after ligating off either the whole or parts of the liver, and expressed the opinion that the disintegration in phosphorus poisoning is identical with autolysis. Umber found the same for absolutely fresh exudates,[7] but of especial importance as bearing upon the present observation is that of Conradi, who showed that during the autolysis of organs substances preventing coagulation appear, and these directly antagonize the action of coagulating

[6] See F. Umber, "Die klinische pathologische Bedeutung der Autolyse," *Berliner klinische Wochenschrift*, 1903, xl, 185. Recently the influence of osmotic changes has found strong supporters in the explanation of cytolysis. See especially Baumagarten," Ergebnisse d. osmologischen Forschung über Haemolyse," in H. Zickel, *Osmolog. Diagnostik u. Therapie.*

[7] This has been extensively studied lately by Opie, *Journal of Experimental Medicine*, 1905, vii, 316, 759.

substances derived from the original organ juice. The latter are of course especially concerned in coagulation necrosis. An extensive application of autolysis to the explanation of pathological processes has been made by Fr. Müller.[8]

Further, according to Jacoby, autolytic ferments are specific for the native albumin of the organ from which they are derived. It is well, therefore, to think of a relation which these experimental chemical observations may have to these anatomical findings.

Apparently little attention has been paid to the morphological changes incident to autolysis. Waldvogel [9] in some recent contributions has paid some attention to the morphology of post-mortem liver autolysis, especially to establish that, as far as the fatty changes in the cells are concerned, great similarity exists between autolysis and phosphorus poisoning.

The fatty changes [10] in all of our cases were quite pronounced. In such disintegrating liver cells fine or coarse fat granules, or drops, appeared early within the fading protoplasm, but without definite regard to location. As the cell disappeared, larger round fat drops occupied the protoplasm, the nucleus was pressed to one side, and an accumulation of similar fat drops was common in the wasted areas. But a marked variation in the morphological fatty changes, as well as in the quantitative distribution, was visible in all the cases, and even in each case; so that, while we have to regard them as a constant associate, no particular characteristics were detected in this respect. The fat in uninvolved liver cells was of the large round drop variety, usually spoken of as infiltration.

Waldvogel's investigations have also shown a great variety of morphological fatty changes in autolysis as well as in phosphorus

[8] See also Flexner: "Note on Autolysis in Lobar and Unresolved Pneumonia," *Transactions of the Association of American Physicians*, 1903, xviii, 1359.

[9] "Autolyse und fettige Degeneration," *Virchow's Archiv*, 1904, clxxvii, 1; "Phosphorvergiftung und Autolyse," *Deutsches Archiv. f. klin. Medizin*, 1905, lxxxii, 437.

[10] In the light of our recent knowledge of fatty degeneration of cells, and the occurrence of lecithin and protagon under such conditions, it will be of interest to pay particular attention to these points in future observations of this kind.

poisoning. In both, drops, clumps, and granules in large numbers occur, and in autolytic livers a large number of masses staining with osmic acid may even be detected within vessels. Waldvogel further regards the macroscopic color of the liver as to a certain extent at least characteristic of the chemical changes: yellowish white indicating a prevalence of protagon, brown a prevalence of jecorin. Our cases showed a diffuse pale yellow as ground color.

It would be of very great interest to compare in detail the various morphological changes in experimental autolytic destruction of cells with our anatomical observations, especially with regard to the occurrence or absence of coagulation necrosis.[11]

Our cases certainly show a cellular destruction unlike that observed in acute yellow atrophy; and none of the associated inflammatory changes of the latter condition are present.

Of particular interest is the bile imbibition of the disintegrating liver cells. It might perhaps be thought that this may stand in causal relation to the lysis of the cells, especially since it has been demonstrated by Salkowsky that the bile possesses such lytic power. Close observation, however, does not bear this out, for not only does the degree of bile imbibition show no relation to the loss of tissue in any of the cases, but there exist also marked individual variations, while the cellular destruction is equally prominent in all four cases.

Investigation shows that the bile is present only in the disintegrating cells, and appears in two distinct types (Figs. 2 and 3): first in the form of fine, small, round, light yellow granules, diffusely occupying the cell, and apparently lying loose on the surface of the disintegrating pale protoplasm. It has all the appearance of a passive invasion. Entirely different is the second, which appears as large, well localized central deposits of bile clumps within preformed vacuoles of the cellular protoplasm. This seems to occur in the better preserved cells, and raises the question whether this form does not present an active secretion of the cell; in that case it would indicate that the

[11] Such observations are now contemplated in this laboratory.

destruction of the cells is a gradual one during which certain of their functions may be retained to a late stage.[12]

Of importance seems the fact that all the lesions occurred in individuals whose general nutrition was lowered, who showed marked degenerative changes throughout, and who had been exposed for some time to unhealthy modes of living.

Clinically the course was that of an intoxication, if we can judge from the somewhat meager histories. In three of the patients delirium prevailed, and in one patient an increasing mental dullness.

The extent of the destruction varied quantitatively: the local and general jaundice, fatty changes, and the amount and distribution of the portal sclerosis with its bile duct proliferation.

It is noteworthy that in a little over a year we have been able to collect four well defined cases.[13] While this may partially be accounted for by the kind of cases received in an institution like ours, it raises the question whether closer attention would not reveal the lesion more frequently. Cases now classified by superficial examination as irregular stasis or parenchymatous degeneration may, if studied more carefully, be found to belong to this category.

It seems as if one would have to regard this affection of the liver as one deserving an independent pathological position, and as representing a definite form of cell degeneration. Further anatomical and experimental investigation will have to determine the clinical and pathological importance and exact position and nature of the lesions.

I desire to express my thanks to Drs. E. Evans, T. C. Janeway, and N. B. Potter for allowing me the use of the cases and the clinical records of them. Dr. B. C. Crowell of the laboratory has been of great assistance to me during the course of this study. The drawings are by Mr. K. K. Bosse.

[12] I have already touched upon the importance of degenerative secretion in "Der primare Leber Krebs," etc., *Virchow's Archiv*, 1905, clxxx, 499.

[13] Among 275 autopsies from October 13, 1903, to February 7, 1905.

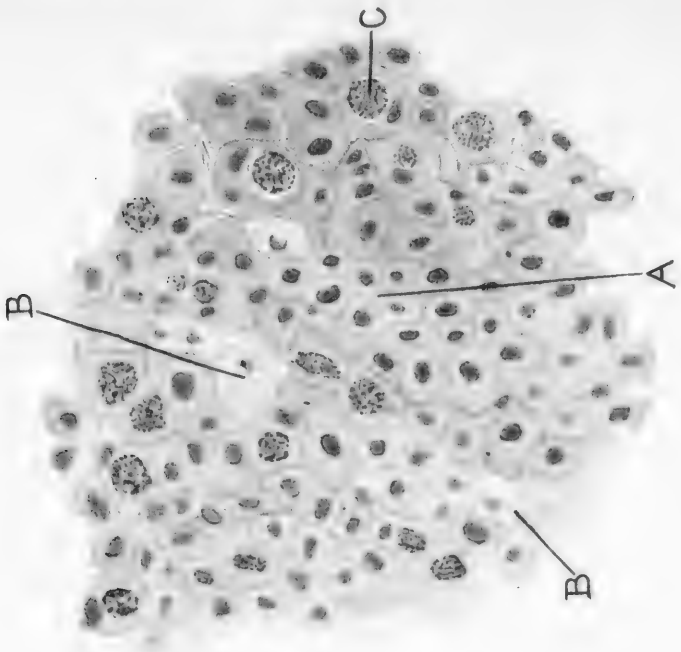

FIG. 3

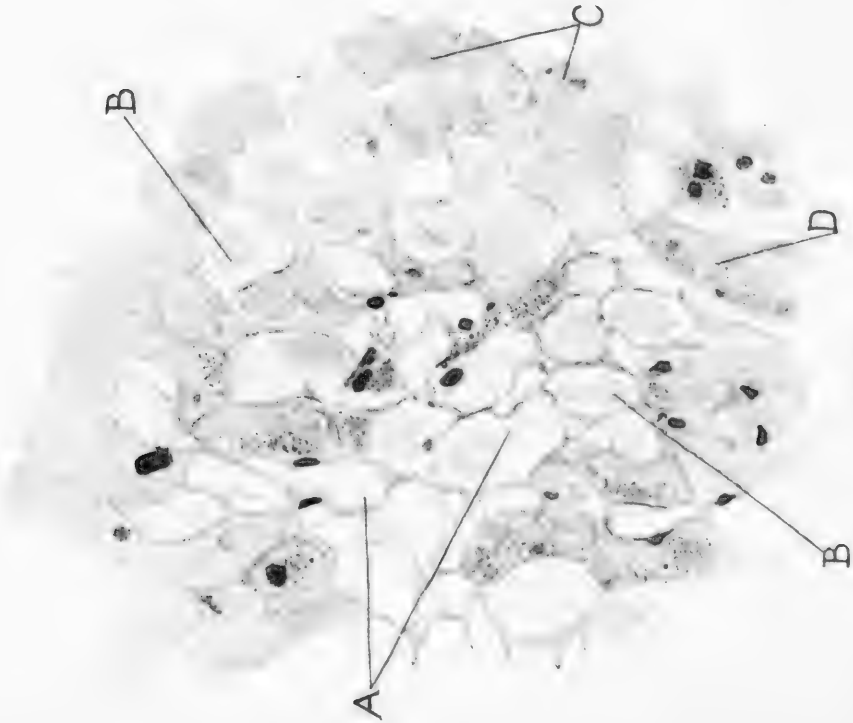

FIG. 2

EXPLANATION OF FIGURES.

Fig. 1.—In text. From Case I.—Leitz obj. 3, ocular 4. General distribution of lesion.

A. Wasted areas showing remaining reticulum with bile granules and cellular remnants.
B. Portal space.
C. Preserved liver tissue with some bile imbibition.

PLATE VIII.

Fig. 2.—From Case II.—Leitz obj. 6, ocular 4. Appearance of a wasted area under high magnification. Hæmatoxylin-eosin stain.

A. Completely lost portion with remaining reticulum and nuclear remnants.
B. Remnants of undissolved but fading protoplasm (cell ghosts).
C. Better preserved cells with beginning solution of protoplasms and irregular cellular swelling.
D. Bile imbibition of disintegrating cells in form of fine granules.

Fig. 3.—From Case III.—Leitz obj. 6, ocular 4. Bile in liver cells. Hæmatoxylin-eosin stain.

A. Normal liver cells.
B. Different stages of early cell destruction.
C. Coarse bile granules in preformed vacuoles.

NEUROMA EMBRYONALE OF THE CHOROID PLEXUS OF THE CAT.

By H. S. STEENSLAND, M.D.

(*From the Pathological Laboratory of the College of Medicine, Syracuse University.*)

PLATES IX AND X.

The examination of the tumor which I am describing was made at the request of Dr. Frank P. Knowlton, who described the physiological features of the animal at the meeting of the American Physiological Society in Philadelphia, December, 1904.

In 1904, Verhoeff[1] described a rare tumor (terato-neuroma) arising from the Pars ciliaris retinæ. He regarded it as of a nature hitherto unrecognized and he discussed its relation to so-called Glioma retinæ. The primary and secondary optic vesicles, embryonic retina, and vitreous humor and the hyaloid membrane were represented in the tumor. Verhoeff thought that the term "terato-neuroma" would seem to express well the general characters of the tumor, indicating as it does a monster-like growth composed of nervous elements, and that the term has the advantage of being applicable to tumors of similar nature which possibly may arise elsewhere in the nervous system.

I shall describe in this paper a tumor of the cat, probably arising in the choroid plexus, which may belong to the same class. In its essential features this tumor appears to represent the central nervous system, although in an atypical and an early embryonic stage, previous to the differentiation of neuroblasts. In a personal communication Verhoeff, who has examined sections of my tumor, suggests the name "Neuroma embryonale" as applicable to both tumors.

The tumor in the cat was situated in the fourth ventricle. In the region of its greatest diameter it occupied the left half of the floor of the ventricle, extending from the median line to

[1] *Trans. of the Am. Ophthal. Soc.*, 1904, x, 351.

the Corpus restiforme. Anteriorly and posteriorly it lay in the depth of the tissue to the ventral side of the ventricle. It was approximately spherical in form and measured in its greatest diameter nine millimeters. The center of the tumor was converted into a large cyst about which the tumor tissue formed a thin wall. In a cross-section of the tumor and surrounding structures a marked disturbance of the internal relations of the medulla and, to a less extent, of the cerebellum was apparent. This was due to the infiltrative growth of the tumor into the tissue, as proven also by the microscopical examination. The tumor consists of tissue resembling the central nervous system in an early stage of development. It shows numerous lumina, in section circular or elongated in form, corresponding to the embryonic neural canal. An inconspicuous mesenchymal framework is present. There is no mesenchymal capsule.

Microscopical examination of the formalin-hardened tumor was made. The Benda method for staining neuroglia fibrils as employed by Huber [2] was used, except that the chloroform method of embedding in paraffin was substituted. Specimens were also stained with hæmatoxylin and eosin, and by Van Gieson's method.

The entire growth is uniform in character. A condition such as that seen in Fig. 4 suggests an early stage of the neural tube, consisting of a single layer of cells or spongioblasts. The cells are not completely isolated from each other so that the tissue is distinctly syncytial in character. The free ends of the cells project through the Membrana limitans epithelii.[3] In Fig. 2 from

[2] Huber, "Studies on the Neuroglia," *Am. Jour. Anat.*, 1901, i, 45.

[3] Dr. Verhoeff proposes this name for the membrane described by him ("A Hitherto Undescribed Membrane of the Eye, and its Significance," *Royal London Ophthal. Hosp. Reports*, 1903, xv, 309), and through his generosity I am permitted to introduce it here. He has also published further observations on the membrane ("The Mixed Tumors of the Lachrymal and Salivary Glands," *Jour. of Med. Research*, 1905, xiii, 319). It is described as a fenestrated membrane, occurring also in the ependyma of the central nervous system, and is compared to a wire screen with hexagonal openings, the latter corresponding in size to the cross-sections of the cells. It corresponds, no doubt, to what is frequently described as the Membrana limitans interna or as a cuticular surface. When seen in cross-section, the membrane gives the appearance of a sharp internal margin to the cells. When seen in plane-section its fenestrated structure is

another part, a later stage of the nervous system is represented in the tumor. The cell boundaries in this part are still less distinct and the neurospongium of His appears to have formed. The dividing nucleus in the internal border zone corresponds to a germinal cell of His. Along the mesenchyma the protoplasm forms a continuous layer corresponding to the Margo limitans. This layer is seen in the figure along two sides, but in some cases, where the tube is seen in section to be completely surrounded with mesenchyma, the picture is even more suggestive of the normal neural tube with its embryonic membranes. The space separating the two represents, perhaps, the epicerebral lymph space, but it is not to be excluded that this appearance may be due to the fixing reagent.

In Fig. 1 a portion of the wall of a tube in a still later stage of development is represented. A section of a blood-vessel corresponding to those which penetrate the normal neural tube at this stage is also to be seen. The Margo limitans is developed about the vessel. The Margo limitans is seen especially well developed below the vessel in the figure, and it appears widely separated from the vessel wall by a space across which pass slender strands of tissue.

The nuclei appear large, vesicular, and round or oval in sections. In the tissues fixed in formalin they have a well-marked chromatin network. The nuclear membrane is distinct and large nucleoli are present (Figs. 1, 2, 3, and 4). In sections stained with hæmatoxylin and eosin the nucleoli appear refractile and translucent (Fig. 1), while with the Benda method they stain intensely black (Figs. 2, 3, and 4). The long axes of the nuclei are usually arranged radially to the lumina. The tissue in the sections appears never to reach a stage where mitosis ceases, and hence mitotic figures are found more or less frequently throughout.

Where the tissue shows later stages of development the sec-

apparent. In the embryonic nervous system the ependymal dots described by Weigert in adult ependyma, where many of them occur in each cell, are arranged in pairs, two occurring in each cell on a level with the fenestrated membrane and in the center of each of its openings.

tions stained by Benda's method show a few neuroglia fibrils, usually at a considerable distance from the lumina. They appear to have the same relation to cells as in normal neuroglia. Nothing corresponding to neuroblasts is seen. Thus a differentiation leading to the formation of neuroglia only, takes place in certain regions.

In serial sections independent lumina can be followed throughout their entire extent. One of the smaller lumina (Fig. 4) is eight microns in diameter measured from the Membrana fenestrata epithelii. The latter membrane is well defined and is seen as a sharp line. The appearance of processes passing out from the membrane between the cells results from the screen-like structure of the membrane. The protoplasm of the spongioblasts appears to project as a continuous mass through the membrane into the lumen. This lumen is evident in only three sections of the series.

It may be that the origin of the lumina is the same as the origin of the lumen in a solid anlage of the nervous system, as in the teleostei. Lumina of intermediate sizes between the smallest and largest are encountered.

Verhoeff speaks in the following way of the possible part played by the Membrana fenestrata epithelii in the development of the lumina in the so-called Glioma retinæ: "The structure of the membrane in the rosettes explains why the latter assume their peculiar form, for it is evident that if a few cells became bound together by such a membrane, and they were otherwise unhampered in their growth, they would be compelled to form a more or less spherical body. If the free ends of the cell groups did not finally meet, a spiral figure would result, and just such spirals are frequently seen. The writer has also found single cells with a membrane on one end, as well as curved groups of several cells which were obviously the beginnings of rosettes. This explains very well why tumor cells are often seen within the cavity of rosettes, evidently being enclosed during the formation of the latter." This method of formation would explain the presence of tumor cells in the lumina in the present case also, but the spiral figures are not met with. Ribbert, in

describing the lumina sometimes occurring in gliomata, notes that some lumina appear to be surrounded with radially arranged cells in only a part of their circumference.[4] The appearance would seem sometimes at least to result from irregularities in the form of the lumina. As a result of this the ependymal layer is cut tangentially in a part of the circumference, and so does not present its best known appearance. In my case, possibly, the membrane has had a more or less spherical arrangement from the time of its origin, the cells in the interior gradually undergoing degeneration and disappearing, but the method of formation of the lumina was not determined.

In Fig. 2 the Membrana fenestrata epithelii is seen to be well defined about a considerable portion of the lumen. Processes of the cells project through it into the lumen. Tumor cells are present in the lumen, a condition which is seen frequently. The protoplasm of these cells appears to have undergone degeneration, staining relatively deeply. The nuclei usually are more or less indistinct. The protoplasm frequently is continuous with the processes of the cells extending into the lumen. The condition, such as that presented in this figure, suggests that cells in the internal border zone, instead of migrating peripherally, may sometimes migrate into the lumina, and then, on account of unfavorable nutritive conditions, gradually undergo degeneration.

In sections tangential to the lumina and passing between the Membrana fenestrata epithelii and the nuclei a further study of the form of the "columnar cells" may be made. The tissue is seen to consist of a syncytium in which there are large spaces (Fig. 3). These spaces correspond to those which, in sections passing through the center of lumina, appear to separate individual "columnar cells" from each other.

The tissue appears to be arranged symmetrically about the lumina in every direction. Nothing corresponding to the dorso-ventral axis of the developing nervous system is seen. The elongated form of some of the lumina suggests a representation of the antero-posterior axis. Sometimes the tubes are folded in a way resembling the folding of the normal neural tube in the

[4] Ribbert, *Geschwulstlehre*, 1904, 336.

development of the various territories of the central nervous system.

In sections tangential to the lumina a study of the Membrana fenestrata epithelii may be made. Pictures corresponding exactly, as far as the membrane and ependymal dots are concerned, to those given in the description of the tumor of the Pars ciliaris, are seen. Where the section passes near the periphery of the lumen the membrane is seen both in cross- and plane-section. The membrane appears to be present in connection with the smallest lumina.

The method of growth of the mesenchymal framework is not evident. No active proliferative process is seen in it. The larger vessels are surrounded by a cellular adventitial sheath which is closely applied to the wall of the vessel and frequently is separated by a wide space from the surrounding epithelium. Often, however, the epithelium is closely applied to the mesenchymal tissue, in which case no evidence of the perivascular space exists.

Origin of the Tumor.—In the region of the fourth ventricle the cells which would seem most likely to possess the potentiality for the formation of the entire nervous system are the epithelial cells lining the choroid plexus. Morphologically, also, they correspond most nearly to the original epithelial cells of the medullary plate and the neural tube. Such an origin would correspond to the origin of the tumor of the Pars ciliaris retinæ, from the unpigmented epithelium of the retina. In sections the epithelium of the choroid plexus may be traced into direct continuity with tumor cells, and transitional forms between the two appear to occur. Pictures are seen corresponding to Fig. 2 in Verhoeff's description, which shows the tumor cells in continuity with the unpigmented epithelium.

In the case of the tumor of the Pars ciliaris it seems probable that the original process was one of resumption by the unpigmented epithelium of properties leading to the formation of the primary and secondary optic vesicles (Verhoeff's Figs. 2 and 8). The condition of the cells in these figures corresponds more or less to those present in my Fig. 4. The lumina in Verhoeff's

tumor arise as the result of a convoluted papillary outgrowth of the epithelium and the underlying stroma. The "second type of convolution," representing the early embryonic retina (Verhoeff's Fig. 6), corresponds most nearly to that shown by my Fig. 2.

In the case of the tumor of the choroid plexus of the cat the epithelium lining the plexus appears to have resumed, although atypically, the properties of the epithelium of the embryonic neural tube.

It may be objected that if the tumor is to be regarded as a neuroma there should be evidence of the formation of neuroblasts. But the tissue may be regarded as in a stage of development anterior to the appearance of these cells. Moreover, there are territories in the lining of the neural tube aside from the one giving rise to the choroid plexus, that give origin only to neuroglia and not at all to nervous elements. This is true, for example, of the territories from which the optic nerves originate. The epithelial cells give rise simply to the neuroglia framework of the nerves which supports the fibers of the nerve cells originating at another level. Yet these cells probably possess the potentiality for the formation of neuroblasts. Perhaps some tumors described as ependymal gliomata belong in the same class with this tumor.

For the drawings of Figs. 2, 3, and 4 I am indebted to Dr. Francis A. Hulst.

EXPLANATION OF PLATES IX AND X.

In all the figures the outlines were drawn with the aid of the Abbe camera lucida (Leitz Obj. 7, Oc. 5), and the detail is added with higher magnification (Leitz Obj. $\frac{1}{12}$, Oc. 5). The drawings, except Fig. 1, are all slightly reduced.

Fig. 1.—A part of the wall of a relatively large tube. Hæmatoxylin and eosin staining.

Fig. 2.—Medium size tube showing the Margo limitans on two sides. The appearance resembles the cross-section of the embryonic neural tube. The stroma is shown to the right and below. Benda's neuroglia fibril stain.

Fig. 3.—Section tangential to a lumen between the Membrana fenestrata epithelii and the nuclei. The relatively deeply staining area of protoplasm in the center of the figure is found in neighboring sections of the series to be associated with a mitotic nucleus. Small amount of stroma shown in lower right-hand corner. Benda's neuroglia fibril stain.

Fig. 4.—Section through the center of one of smaller tubes. The appearance similar to that of the neural tube consisting of one layer of cells. Benda's stain.

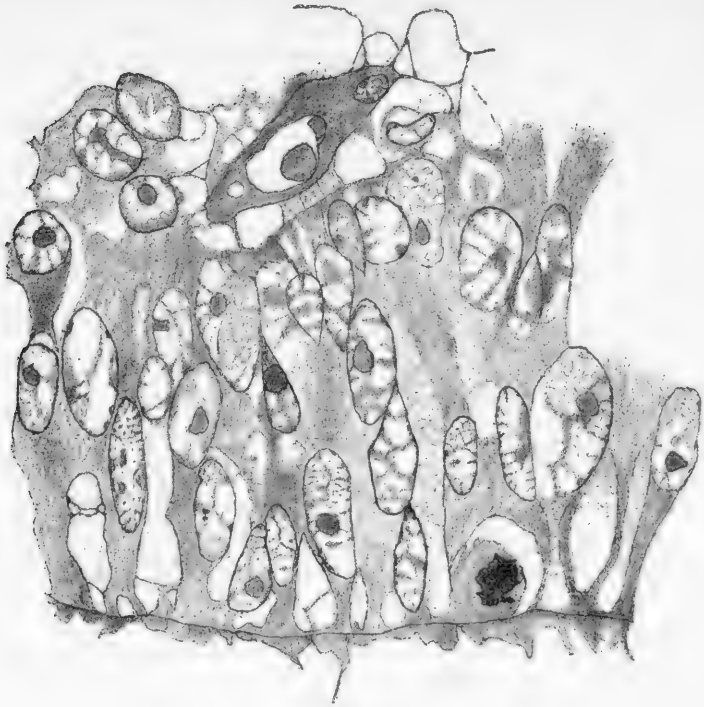

FIG. 1

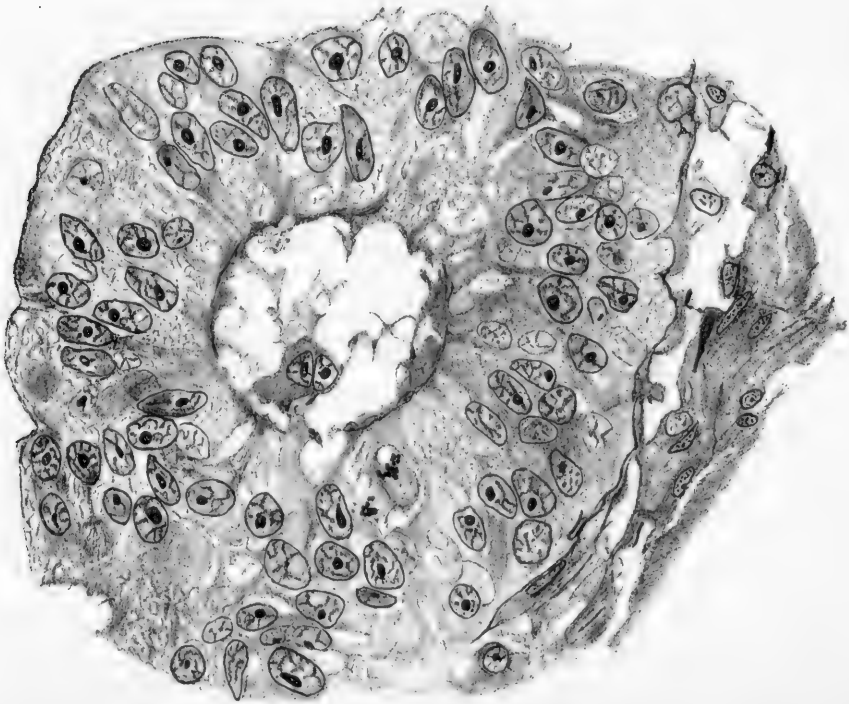

FIG. 2

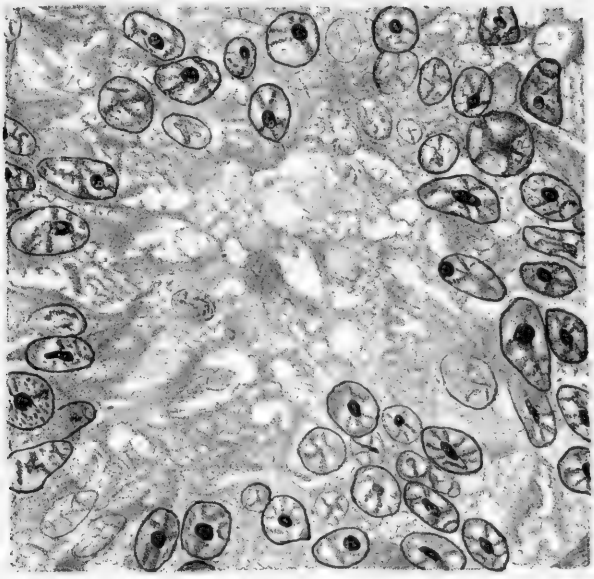

FIG. 3

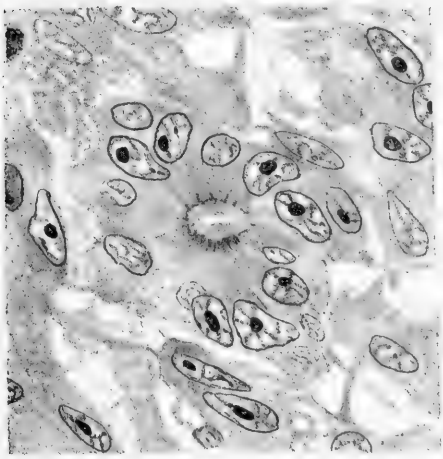

FIG. 4

STUDIES ON THE TOXICITY OF BILE.

II.

THE TOXIC EFFECTS OF BILE UPON THE CENTRAL NERVOUS SYSTEM AND THE ELIMINATION OF STRYCHNINE THROUGH THE BILE IN NEPHRECTOMIZED ANIMALS.

By S. J. MELTZER and WILLIAM SALANT.

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The occurrence of severe nervous manifestations in the course of diseases in which icterus is a predominant symptom gave rise at an early period to experimental investigations of the effect of bile upon the animal organism. During the first part of the last century the methods employed in these investigations consisted mainly in intravenous injections of ox bile obtained from the gall bladder. As in most of the experiments the injections caused the death of the animal, it was ascribed to the toxic effect of the bile. During the forties of the last century, however, the view gained ground that death following intravenous bile injections was not due to the toxicity of the bile, but to impurities in the fluid used which give rise to the formation of capillary thrombi in vital nerve centres. Thus Bouisson reported that animals which received filtered bile survived the intravenous injections without manifesting any important nervous symptoms. This result could not have failed to exert a deciding influence upon contemporary opinion, and we need not therefore be surprised to find Henle [1] making the statement that the widespread view of the toxicity of bile was an unwarranted assumption. However, after the discovery by Strecker [2] of bile acids, numerous investigations were carried out with them, and any question of accidental causation of thrombosis could therefore no longer

[1] Henle, *Handbuch der rationellen Pathologie*, 1847.
[2] A. Strecker, *Habilitationsschrift*. Giessen, 1848.

enter seriously into the discussion of the results. The first extensive study of the toxicity of bile salts was made by von Dusch.[3] And among the subsequent investigators we find such well-known names as Frerichs, Leyden, Kühne, Traube, Hoppe-Seyler, Landois, and many others. The last investigation of this subject, so far at least as the effect upon the nervous system is concerned, was carried out some fourteen years ago under Kobert's direction by D. Rywosch.[4] It was a comprehensive, painstaking piece of work which has left its impression upon all later writings on the subject of the toxicity of bile.

The present status of the views entertained concerning the toxicity of bile may be described as follows: All agree that bile is a very toxic substance. It hæmolyzes red blood corpuscles and dissolves white blood corpuscles and other tissue cells; it retards and stops the heart-beat, lowers the blood pressure, and is capable of affecting the structure of nerve and muscle, etc. Regarding the effect upon the central nervous system, there is unanimity of opinion that bile produces depression; most of the investigators describe stupor and weakness of the animals as the predominant symptoms following the intravenous injection of bile salts in mammals, and coma and paralysis following their injection into the lymph-sacs of frogs. A few investigators have also observed occasionally the occurrence of convulsions. von Dusch [5] describes three experiments in which the animals died in convulsions a few minutes after receiving intravenous injections either of filtered bile or of sodium cholate. Röhrig [6] observed similar effects in two experiments in which 7 c.c. of a 5 per cent. solution of sodium cholate were injected into the jugular vein. Leyden [7] described a few experiments in which convulsions appeared after injection of bile salts into the jugular vein; in one case the convulsions appeared immediately after injection of one c.c. of a 10 per cent. solution of sodium glycocholate. In the vast literature on the studies of bile there are scattered records of a few more experiments in which convulsions occurred among the effects of the injection of bile. However, even Leyden, who more than any other author insisted upon the appearance of convulsions after intoxication with bile, states that after all it is a rare occurrence. We [8] have ourselves stated in a previous paper that in most experiments the convulsions set in soon after the intravenous injection of bile or bile salts, and have expressed the view that they were simply terminal manifestations due to heart failure produced by too rapid injection of the solutions. In a few exceptional cases convulsions are recorded to have

[3] von Dusch, *Habilitationsschrift*. Leipzig, 1854.
[4] D. Rywosch, *Inaugural Dissertation*. Dorpat, 1891.
[5] von Dusch, l. c.
[6] Röhrig, *Dissertation*. Würzburg, 1863.
[7] Leyden, *Beiträge zur Pathologie des Icterus*. Berlin, 1866.
[8] Meltzer and Salant, *Journal of Experimental Medicine*, 1905, vii, 280.

developed a day or two after subcutaneous or intraperitoneal injections. Since, however, in these instances the bile or bile salts tend to produce extensive local necrosis, the convulsions are not necessarily to be regarded as due directly to the bile; they may have been caused by absorption of toxic products from the necrotic areas, or the result of a terminal infection. In frogs the toxic symptoms appear so soon after the injection of bile or bile salts into the lymph-sacs as to be ascribed directly to bile intoxication. Leyden,[9] Loewit,[10] and Rywosch [11] tested bile salts upon frogs, and report that coma and paralysis are the only effects which they have observed. Rywosch indeed asserts that in all his experiments, in which mammals were also included, he never saw anything but a depressing effect. It was this last statement which caused the more recent writers on diseases of the liver to abandon the old theory of cholæmia as due to bile absorption in acute yellow atrophy of the liver or of any other form of *icterus gravis*. The symptoms are sometimes of a depressing character, as in coma, etc., and sometimes manifestations of increased excitability, as in convulsions or excitation mania. On account of the occurrence of the latter symptoms, Stadelman [12] and others insisted that bile absorption could not be the cause, and they argued that bile causes only coma and similar symptoms of a depressing nature, but never convulsions.[13]

To sum up briefly, it may be said that bile, according to the present almost universally accepted view, contains a toxic element which is capable of producing nervous depression, coma, and paralysis, but does not contain any element which can produce excitation and convulsions.

Our experiments have consisted in the injection of bile and bile salts into animals. We have employed chiefly rabbits' bile, although bile from the ox, dog, and guinea-pig was also used. In addition to this, experiments with glycocholate and taurocholate of sodium were made. The frog was chiefly employed for the injections, which were made into the dorsal or ventral lymph-sacs. There are many reasons for selecting frogs for the experi-

[9] Leyden, l. c.

[10] Loewit, *Zeitschrift für Heilkunde*, 1881, ii, 459.

[11] Rywosch, l. c.

[12] Stadelman, *Ueber Icterus*. Stuttgart, 1891.

[13] The recent observation by Biedl and Kraus [14] that subdural injections of bile cause convulsions can hardly be taken as a valid proof that bile contains a tetanizing element, since even minute doses of such comparatively innocent substances as methylene blue, which by intravenous injection are almost harmless to the animal organism, can produce convulsions when injected subdurally or intracerebrally.[15]

[14] Biedl and Kraus, *Centrabl. f. klin. Med.*, 1898, p. 1185.

[15] Bruno, *Deutsche med. Wochensch.*, 1899, xxv, 369.

ments, for, from what we have stated above, it is evident that mammals are not very suitable for them. When in mammals the injections are made intravenously, the rapid reactions which often follow are due to the effect of the bile upon the heart, and not to a primary effect upon the nervous system. When, on the other hand, the injections are made into the peritoneum, etc., the reactions sometimes set in so late as again to permit of doubt as to whether they are or are not primary toxic effects. The case with frogs is different. The reactions which follow the injections of bile into the lymph-sacs set in early enough to be due directly to the bile intoxication; nor can there be any suspicion that the nervous manifestations are secondary to some disturbance in the circulatory or respiratory systems.

As the main title of this paper indicates, the chief part of this research was devoted to a study of the toxic effects of bile upon the central nervous system. This, however, was not the primary consideration which started us on this long investigation; for, indeed, we were in quest of a solution to a problem which presented itself to us in the course of our previous researches. Before the latter could be attacked, we became confronted with the question of the normal toxicity of bile, in the study of which new thread we arrived at results which shed light on this new problem, while, at the same time, it assisted in elucidating the original problem. And herein will be found the chief reason for using rabbits' bile upon frogs. In presenting our results it would be natural to follow the tortuous path by which we gradually arrived at them. We shall therefore begin with the statement of the original problem which started us on this research.

It is an established fact that strychnine when introduced into the body by any path leaves it entirely through the kidneys. We[16] have, however, found that after double nephrectomy, two or three times the fatal dose of strychnine can be injected without causing intoxication, provided each single quantity injected is less than the minimum toxic dose, and the intervals between the injections are not too short; that is, it appeared that after nephrectomy there is no cumulative effect of the strychnine in the

[16] Meltzer and Salant, *Journal of Experimental Medicine*, 1901–1902, iv, 107.

blood. One of the possible explanations for this remarkable condition lies in the assumption that after nephrectomy the process of elimination is assumed by another organ. The gastro-intestinal canal suggested itself to us as this channel,[17] but we failed to detect strychnine in it. Later, [18] it was found that the methods at hand for detecting traces of strychnine make it difficult to discover small quantities of it in the intestinal canal. Hence, in the further pursuance of the question of vicarious elimination, we turned our attention to the bile through which, it is claimed, poisons are normally eliminated from the body. Our problem therefore was to determine whether, after nephrectomy, strychnine is eliminated through the bile. As our previous experiments were made on rabbits, it was necessary therefore to make the new ones on the same animal. Hence our reason for using rabbits' bile in this investigation. Furthermore, as the chemical method for separating strychnine from bile is very tedious, and would become quite unmanageable if, as in our plan, a large number of experiments were to be performed, we resorted to the biological test—i. e., the injection of bile supposed to contain strychnine into frogs in order to produce the usual well-known strychnine convulsions.

In attempting to solve this problem several series of experiments were made. In one series the rabbits which were nephrectomized had their common bile duct ligated. After recovery from the operation the animals were given several subminimum doses of strychnine. The animals were studied first with reference to the amount of strychnine they could stand without suffering a convulsion, and second, after the death, in that the bile from the gall bladder and the dilated duct was injected into frogs. In another series of experiments a cannula was tied in the common duct of the nephrectomized rabbits, and, after injecting subcutaneously subminimum doses of strychnine, the collected bile was tested on frogs. The following is a brief *résumé* of these experiments.

Series I.—*Both kidneys removed, the common bile duct ligated,*

[17] Salant, *American Medicine*, 1902, iv, 293.

[18] Salant, *Journal of Medical Research*, 1904, xii, 41.

and, subsequently, subminimum doses of strychnine injected at varying intervals.

This series included seven rabbits. Four showed no signs of a cumulative effect, although the sum total of the several doses of strychnine in each animal exceeded by far the minimum fatal dose (about 0.6 milligramme per kilo). One animal lived 74 hours and received during that time nearly 3 milligrammes per kilo without showing either convulsions or hyperæsthesia. It died under the symptoms of exhaustion.

The behavior of these four animals was identical with that of nephrectomized rabbits in which the common duct was not ligated.

Of the remaining animals, one received within twelve hours about 0.9 milligramme per kilo of strychnine without showing any effects. Three hours later, however, when it was again given about 0.2 milligramme per kilo, the animal had a tetanus and died in a few minutes. The course in this experiment did not differ materially from that which sometimes occurred in our previous experiments on nephrectomized animals without ligation of the duct, which received strychnine in refractive doses.

The remaining two animals, however, died in violent convulsions two hours after receiving only 0.3 milligramme of strychnine per kilo, a dose which is, as a rule, non-toxic even for normal animals.

These observations, then, did not permit of any positive conclusions being drawn one way or the other. In the majority of the animals the closing of this additional eliminating path did not increase in the slightest the cumulative effect of the poison within the blood, a fact which would speak against the elimination of the strychnine through the bile. On the other hand, in two experiments, ligation of the bile duct did produce a rather striking aggravating effect upon the injection of a single subminimum dose of strychnine. Furthermore, we had to take into consideration that the absence of strychnine symptoms in the majority of these animals could not be taken as an absolute proof of the absence of strychnine in the bile, because it was possible that the refractive doses of strychnine were eliminated from the blood by, and accumulated largely in, the bile within the gall bladder, but through the abnormal state of the bladder under these conditions the strychnine was prevented from being re-absorbed into the circulation, which would account for the absence of convulsions in most of the experiments.

In the latter case we would expect that the bile of these

animals when injected into frogs would produce symptoms of strychnine poisoning. This view was tested. Bile was taken from the gall bladder and the dilated bile duct from four rabbits of this series. The quantities were of course small and only a few experiments could therefore be made. The few results were as follows:

One c.c. of the bile injected into a medium-sized frog caused coma in one hour; and ½ c.c. caused in a small frog complete coma and paralysis half an hour after injection. As to the remainder, it can be briefly stated that none of the frogs which received smaller or larger doses of the bile showed convulsions or hyperæsthesia.

These few observations did not indicate that the bile contained strychnine, at least not in such quantity as to set up characteristic symptoms in frogs.

Regarding the preceding experiments it may be said that they were capable of providing positive proof only if all the animals had manifested strychnine poisoning, or in other words if the bile of the animals had contained strychnine we should have had evidence that after nephrectomy strychnine is eliminated in this secretion. However, the failure of these experiments to bring forward this proof can not be interpreted as disproof, namely, that the biliary organs do not assume the function of elimination of strychnine after nephrectomy; for, just as we have assumed that after removal of the kidneys another organ, the liver, takes over the function of elimination, we may now assume that after nephrectomy and ligation of the common duct still another organ assumes the work of elimination of strychnine; whence the failure of the above experiments to show that the bile contained strychnine. We expected to obtain more satisfactory evidence regarding this assumption from the following set of experiments.

Series II.—*Rabbits were nephrectomized and a cannula placed in the bile duct; strychnine in subminimum doses injected, and the bile collected and introduced into frogs.*

We made six such experiments. In five animals some bile was collected before the strychnine injections were given. This bile was also injected into frogs which served as controls. All the frogs receiving bile from the ante-

strychnine period responded with coma and all but one became paralyzed and died. None of the frogs showed tetanus, rigidity, or convulsions, and all but one showed even no hyperæsthesia. One showed marked hyperæsthesia, but soon also passed into coma and paralysis.

The frogs which received the bile from the strychnine period presented a different picture. The bile from one rabbit which received within six hours 0.7 milligramme of strychnine nitrate caused in three frogs tetanus and death in twenty minutes. The bile from another rabbit which received 2 milligrammes of strychnine in forty-eight hours produced tetanus in two frogs, marked hyperæsthesia in one, and had no effect in another; in the last case a dose of bile as large as three c.c. was given. Two rabbits were given about 0.3 milligramme of strychnine; the bile of one produced tetanus in one, marked hyperæsthesia in another, and coma and paralysis in a third frog. The bile of the other produced late rigidity and convulsions in one and coma in another frog. Of the remaining two rabbits, one received 0.5 milligramme of strychnine in a single dose; the bile of this animal caused in one frog hyperæsthesia which terminated in coma, and in another it produced only coma. The same effect resulted from the bile of the last rabbit, which received a single dose of strychnine (the amount is not noted in the protocols).

The bile (strychnine period) from the rabbits of this series produced distinct strychnine symptoms (hyperæsthesia, etc.) in nearly all the frogs, while distinct tetanic attacks were produced by the bile of rabbits which received at least toxic doses. Comparing these results with those obtained with the bile from the ante-strychnine period of the same series, the conclusion seems to be justified that in these animals some of the strychnine at least was eliminated through the bile.

New questions now arose. In the first place, the question as to whether the presence of strychnine in the bile of this series of animals represents a vicarious process only, and whether in normal animals which receive strychnine the bile does not also, as a rule, eliminate part of the poison; and in the second, whether the bile of nephrectomized animals, not receiving strychnine, does not contain a tetanizing element of metabolic origin, which may have caused the hyperæsthesia and convulsions described in the last series of experiments. These questions were tested by short series of experiments.

Series III.—*Bile collected from a cannula in the common duct of normal rabbits before and after the injection of strychnine and tested on frogs.*

Only three such experiments were made. One animal received 0.9 milligramme of strychnine. Two c.c. of the ante-strychnine bile of this animal gave coma which developed gradually within one hour. Two c.c. of the bile of the strychnine period gave marked hyperæsthesia, and the addition of two c.c. more of the bile brought out immediately a violent tetanus which passed over rapidly into coma and paralysis.

In the second experiment the rabbit received one milligramme of strychnine. Two c.c. of the ante-strychnine bile gave coma as before, whereas the same amount of bile of the strychnine period gave pronounced hyperæsthesia, which after an hour passed over suddenly into coma (a very brief period of convulsions might have been overlooked). In both the experiments the urine collected after the administration of strychnine was also tested; it was almost without effect.

The third animal received 1.5 milligrammes of strychnine. Two c.c. of the bile (before and after strychnine injection) produced no effect. Three c.c of the bile of the strychnine period produced coma, while three c.c. of the bile of the ante-strychnine period produced rigidity of the frog. (The urine of this animal caused increased reflexes in frogs.)

Hence we have among this series two experiments in which the bile from non-nephrectomized rabbits, after injection of strychnine, produced marked hyperæsthesia and even a convulsion, while in the third experiment the normal bile of the rabbit (before receiving strychnine) produced symptoms distinctly similar to those of strychnine poisoning.

Series IV.—*Bile collected from the common duct of nephrectomized rabbits and tested on frogs.*

We had five such experiments. In one even three c.c. of the bile produced no effect upon the frogs, while three and a half and five c.c. brought on prolonged tetanic attacks. In another experiment three c.c. had no effect, and five c.c. produced hyperæsthesia, but neither convulsions nor coma. In a third experiment five c.c. brought on hyperæsthesia and stupor. Of the bile of the fourth experiment three c.c. had no effect, but five c.c. brought on coma and paralysis. Finally, in the fifth experiment two c.c. of the bile produced coma and paralysis.

In this series, with entire absence of strychnine, marked hyperæsthesia was a frequent phenomenon, and distinct tetanic attacks occurred. On the other hand, coma and paralysis were seen only in two instances and in one instance only after injecting five c.c. of the bile.

A remarkable fact of this series is that in nearly all of the experiments as large doses of bile as three c.c. produced no effect at all.

The control bile of all the previous experiments (except one) produced with two c.c., and sometimes even with one c.c., distinct coma and paralysis. The ante-strychnine bile in the second series was also obtained from nephrectomized rabbits and is therefore comparable with the bile of the last series in every respect except one, that it was obtained at the very beginning of the flow of bile from the cannula. This latter fact might indeed be an important feature and perhaps explain the absence of hyperæsthetic and convulsive effects from the bile of the ante-strychnine period of the second series. We may, perhaps, assume that the hypothetical tetanic element, which normally is eliminated through the urine and after nephrectomy finds its way out of the body through the bile, appears in the bile in effective doses only a time after the removal of the kidneys —that is, that the vicariation develops only gradually. The bile of the fourth series was collected during a long period, sometimes extending over two days and longer, after the nephrectomy, whereas the bile of the ante-strychnine period of the second series was collected only an hour or two immediately following the nephrectomy. Hence, the absence of a tetanic element in the one and its frequent occurrence in the other bile.

However this explanation may account satisfactorily for the production of hyperæsthesia and convulsions in the experiments with the bile of the fourth series, it does not explain the remarkable reduction in toxicity. What could the factor be that caused the surprising reductions in the elements producing coma and paralysis in the bile of the fourth series so that even such quantities as three c.c. had no effect?

Could the presence of a tetanic element in the bile, be it strychnine or some catabolic product, influence the activity of the elements producing coma and paralysis normally present in the bile so as to neutralize to some degree the depressing effect?

Before this question could be properly disposed of, a more fundamental proposition confronted us and had to be studied, namely, as to what the actual effect of the bile collected for a longer period from the common duct of a normal animal is. In

the preceding series we had not yet dealt with this question. The control bile of the third series, which was a normal bile, was collected only for a short period and soon after the introduction of the cannula into the bile duct, in which instances the possible after-effect of the anæsthesia had to come into consideration. But even in this short series the results were not uniform. The control bile of the three experiments produced coma and paralysis in two and rigidity in one animal.

In reviewing the literature of this subject we learned that the opinion now generally held is that bile produces coma and paralysis. It would seem as if only three investigators experimented upon frogs. Leyden made injections in three, and Rywosch in four frogs, while Loewit, who probably made many injections, states his general conclusions only. The three investigators employed chiefly biliary salts, or when bile was used it was ox bile obtained from the gall bladder. It has been generally assumed that bile obtained from the gall bladder is more poisonous than that obtained from the common duct. It is therefore evident that there has been as yet no information whatsoever secured as to the behavior of bile collected from the common duct of normal rabbits. We have studied this subject by means of a large number of experiments, and will now proceed to report briefly the results obtained from them and from others made with stagnant bile from the gall bladder and the dilated, ligated ducts of otherwise normal rabbits.

Series V.—*Bile of normal rabbits.* a. *Bile from the gall bladder or from the dilated, ligated duct.* b. *Bile collected from a cannula in the common duct.*

a. We made only a moderate number of these experiments. The results were as follows. As small quantities as one-half c.c. produced no immediate effect, but most of the animals were found dead the next day. One c.c. produced, as a rule, coma and paralysis an hour or two after injection. In two instances even one and a half c.c. produced only a moderate effect which set in late. (It seemed that injections into the ventral brought out the effect more promptly than into the dorsal lymph-sac. Possibly the abdominal pressure in the normal posture acts like massage and hastens absorption.) In no case was any increase of reflexes, or convulsions, or rigidity observed.

b. As a rule large quantities of bile were collected in this manner, sometimes more than 100 c.c., and thus experiments could be performed upon many

frogs with the bile obtained from one animal. The data which we give were gathered from experiments made with bile of nine different rabbits. In many of the numerous experiments the employment of the normal bile was a part in some experiment in which the normal bile served as a control for comparison with the effect of bile of some other kind.

The chief result of this series of experiments is the establishment of the fact that the bile of some rabbits can produce distinct hyperæsthesia and tetanic attacks. Whether the bile is moderately or strongly toxic, and whether the predominating toxic effect is depressing, as in coma and paralysis, or exciting, as in hyperæsthesia and convulsions, seemed to depend greatly upon the individuality of the animals. Our experience with these animals and with other experiments in the course of this research, led us to feel justified in dividing rabbits into three groups according to their individualities as above indicated.

The bile of one of the groups caused coma and paralysis essentially. Two c.c. would, as a rule, produce these symptoms within a few hours, and the larger the quantity injected the sooner would they set in and the more complete would they be. Quantities less than this but above one c.c. produced during the eight or ten hours of observation scarcely any perceptible effect, but on the next day or the third day the animals would be found dead. One c.c. of bile was almost without action. The bile of these animals produced neither hyperæsthesia nor convulsions no matter what the amount given. We wish to emphasize that the bile of this group of animals produces coma and paralysis but never hyperæsthesia and convulsions.

In a second group of animals bile in quantities of two c.c. or even two and a half c.c. produced no effect whatever. Three c.c. of bile caused sooner or later more or less marked hyperæsthesia, from which the majority of the frogs recovered. Doses of five c.c. produced symptoms of coma and paralysis more or less distinct. As the doses were increased, the symptoms intensified and appeared the sooner. These animals all succumbed. The main characteristic of this bile is that small doses produce hyperæsthesia and larger ones coma and paralysis. In exceptional cases coma would be preceded by a convulsive attack of

short duration and easily overlooked. On this account we may have missed certain of their manifestations.

The bile of the third group in doses of from two to three c.c. produced at most no effect. Three and a half c.c. or more produced marked hyperæsthesia passing into clonic or tonic convulsions. Sometimes a violent tetanus would set in two or three hours after injection of the bile, and at other times the tetanic effect appeared the next day, or only after prolonged handling of the frogs; it tended to be of a pronounced tonic character, the animal remaining uninterruptedly rigid for two minutes or longer. Again the tetanic effect appeared only after such large doses as six, seven, or more c.c. Some frogs survive mild tetanic attacks and return to a good condition. Those which had violent or prolonged tetanic attacks did not, as a rule, recover, but behaved as if poisoned with a toxic or fatal dose of strychnine. None of the frogs which received bile of this group of animals ever showed coma and paralysis.

The bile of the first or coma-producing group was toxic in small doses; the bile of the third or convulsive group exerted a toxic effect only in larger doses; the bile of the intermediate group, producing hyperæsthesia in smaller, and coma in larger doses, possessed only moderate toxicity.

In no instance did we encounter a bile which in small doses caused coma and in larger ones convulsions or hyperæsthesia.

These experiments established beyond doubt that the bile of some rabbits contains a pronounced tetanic element. In certain specimens of bile the tetanic constituent was the only noticeable toxic agent, in others the depressing toxic element existed in moderate amount, the tetanic effect manifesting itself only by hyperæsthesia and in those doses which did not exert a depressing effect. In one kind of bile only could no evidence of the presence of a tetanic element be discovered, namely, in that which in small doses exerted a strong depressive action leading to coma and paralysis.

The question which now arose was this: Does the bile which produces only coma and paralysis actually contain no tetanic element? From the behavior of the bile of the intermediate

group we had reason to believe that the element which produces coma and paralysis, when present in sufficient strength, is capable of suppressing the manifestations of the tetanic constituent. It is therefore conceivable that the very depressing bile of the first group contains a tetanic body, the manifestation of the action of which is rendered impossible because of the fact of the suppressive action of the depressing element.

In following out this possibility we succeeded in demonstrating the presence of a tetanic element in the bile of all the rabbits studied.

We had observed that a medium dose of bile of the intermediate group was capable of bringing out hyperæsthesia although the bile surely contained a certain amount of the depressing element. The reason for this result apparently rested on the fact that in this dose of bile, which was subminimum for the depressing effect, the mutual relations between the tetanic and the depressing substances were in favor of the tetanic agent. We now thought that by artificially supporting by strychnine in ineffective doses the tetanic element, when present in the bile in subminimum doses, it might also be possible to develop the hyperæsthesia and perhaps even the convulsions.

In a previous part of this paper we were led to consider whether strychnine or a tetanic substance of catabolic origin might not be capable to some extent of overcoming or neutralizing the depressing effect of the coma and paralytic elements. Stimulated by these questions, we carried out several series of experiments in which the effects of bile supported by additional injections of strychnine were studied.

Series VI.—*Bile and strychnine injected into frogs.*

In a few experiments strychnine was mixed with the bile in certain proportions and kept for some time before injection. In most of the experiments, however, bile and strychnine were injected consecutively in different parts. The doses of strychnine were known from many previous experiments to produce either no effect or a very slight one; besides which facts, in many of the tests the dose employed was carefully controlled.

At the outset it will be well to state that in all cases the addi-

tion of a subminimum dose of strychnine brought out perceptible effects which were not seen in the control experiments with strychnine or bile alone.

Group I.—Bile from nephrectomized animals and strychnine were mixed in proportion of 1 : 10,000, kept for 24 hours, and then injected into frogs.

Small frog A, control; injected into d. l. s.* 0.01 mgr. strychnine nitrate at 3.17 P.M. Observed until 5 P.M.; no increase of reflexes. Next day normal.

Frog A′ size of control; injected into d. l. s. 0.1 of a mixture of 0.1 bile + 0.01 mgr. strychnine at 3.22 P.M.; 4.07 reflexes distinctly increased; 4.15 violent tetanus. Next day again normal.

Small frog B, control; injected into d. l. s. 0.02 mgr. strychnine nitrate at 11.23 A. M. At 12.15 P.M. no increase of reflexes; 12.55 reflexes slightly increased; 1.10 on handling, a mild tetanic attack which soon passed over.

Frog B′ size of control; injected into d. l. s. 0.2 of a bile mixture of 0.2 bile + 0.02 mgr. strychnine nitrate at 11.28; 12.04 violent tetanus.

Large frog C, control; injected into d. l. s. 0.05 mgr. strychnine nitrate at 11.52 A.M. 12.53 P.M. no increase of reflexes; 1.15 reflexes increased; 2.10 reflexes markedly increased; 2.22, violent tetanus.

Frog C′ size of control; injected into d. l. s. 0.5 of a bile mixture of 0.5 bile + 0.05 mgr. strychnine nitrate at 12 M. 12.40 P.M. marked increase of reflexes; 12.53 violent tetanus.

The presence of a tetanic element in the bile in this group of experiments is quite evident. The doses of bile employed never alone produced hyperæsthesia, to say nothing of tetanus. Nevertheless one sees in the three experiments a marked increase in the efficiency of the strychnine produced by the addition of the bile.

Group II. Frog A, control; injection of 0.01 mgr. strychnine nitrate. No effect. Frog A′ injected 0.01 mgr. strychnine + 1.5 salt solution (0.85 %). After 3½ hours very slight temporary increase of reflexes (?). Frog A″, 0.01 strychnine + 3 c.c. salt solution. After 3½ hours slight temporary increase of reflexes (?). Frog A‴, 0.01 mgr. strychnine + 5 c.c. salt solution; same as the others. All practically normal.

Frog B, injected 1.5 c.c. bile from common duct of a normal rabbit at 10.30 A.M. Condition normal; next day the same. Frog B′, injected 1.5 c.c. same bile as above and immediately after injected 0.01 mgr. strychnine nitrate at 10.30 A.M. At 2 P.M. reflexes markedly increased, and at 2.10 on handling, tonic rigidity. At 6.30 reflexes still increased. Next day frog perfectly normal.

Frog C, injected 3 c.c. of the above bile at 10.30 A.M.; at 2 P.M. increased reflexes; soon coma; at 6 P.M. dead.

* The abbreviation d. l. s. will be employed to designate the dorsal lymph-sac.

Frog C′, injected 3 c.c. bile and immediately after 0.01 mgr. strychnine nitrate. At 2 P.M. increased reflexes; no coma; at 6.30 P.M. reflexes markedly increased and no coma. Next day animal perfectly normal.

Frog D, injected 5 c.c. of normal bile; at no time increased reflexes; coma and paralysis soon appeared, and later in the day the animal was found dead.

Frog D′, injected 5 c.c. of normal bile at 11 A.M.; animal soon drooped; injected 0.01 mgr. strychnine. One hour after the injection of strychnine a tetanus broke out; at 6 P.M. animal still tetanic. Recovered next day and remained perfectly normal.

This group of experiments is very instructive. Doses of strychnine, the ineffectiveness of which was established in many controls, brought out distinct specific symptoms when given with bile, and with the increase of the dose of bile, although the dose of strychnine remained the same, the strychnine symptoms increased in intensity, so as to bring on a distinct tetanic state, lasting many hours. This result can only mean that normal bile contains a tetanic element or a substance which in its effect is similar to that of strychnine.

Moreover, this experiment demonstrates a still more important fact. In C and D doses of three and five c.c. of normal bile produced coma, paralysis, and death, whereas in C′ and D′ by the addition of a subminimum dose of strychnine not only was a tetanus brought out, but coma and paralysis were also prevented and the animals survived the fatal dose of bile.

It will be recalled that the bile from the common ducts of certain rabbits produced, in comparatively large doses, hyperæsthesia and convulsions. This result means that in these doses the tetanic element is present in at least minimum toxic doses.

The bile of some other rabbits causes in comparatively small doses coma and paralysis. We have demonstrated that even this bile contains the tetanic element. That this element fails to manifest its presence when injected without the addition of strychnine is probably due to the overpowering influence of the depressing element of this bile. Some of the cited experiments show, and it will be shown more fully later, that the depressive substance in bile, when present in effective strength, is capable completely of effacing the effects of a tetanic element, be it strychnine or a substance of metabolic origin.

It will be recalled also that the bile of some rabbits could be injected into frogs even in quantities of three or four c.c. without toxic effect of any kind. The explanation of this fact may be that in these specimens none of the toxic agents was present in such strength in the doses mentioned as to have a toxic influence. We have, however, reason to assume that in at least some instances the tetanic elements were strong enough in the doses employed to produce hyperæsthesia and convulsions. The harmlessness of this bile may, however, have been due to the simultaneous presence of the depressing element in quantities sufficient to neutralize the tetanic effects. It has been shown above that in some cases the dose of the bile alone would produce coma, but when a subminimum dose of strychnine was added to it the effect might be a tetanus. Hence the tetanic element of the bile plus the subminimum dose of strychnine were capable of antagonizing to such a degree a fatal dose of the depressing element as to produce a toxic effect in the opposite direction. We can under these circumstances easily conceive that were the strength of the tetanic element in the bile slightly less, the result would be a simple neutralization, and neither coma and paralysis nor tetanus be developed but the frogs would have appeared simply normal.

These neutralizations might occur in many ways. There might be, for instance, a certain kind of bile which in a certain dose contains exactly a minimum dose of the depressing element; the result would be coma and paralysis were it not for the simultaneous presence of the antagonistic tetanic element in a strength weak but sufficient to neutralize a fraction of the depressing element, thus reducing it to an ineffective strength. Or the tetanic element might be present in an exactly minimum strength and the depressing element in a fraction of a miminum dose. Neutralizations might perhaps also occur when both antagonistic elements are present in minimum doses or above them, provided they are present in such proportions as to be exactly sufficient to neutralize one another. As a rule, however, as will be seen later, such mixtures as the last do not lead to neutralization.

It should be stated that the cases in which the paralyzing effect of three and five c.c. of normal bile was completely overcome and turned into the opposite effect by the addition of strychnine, were not of frequent occurrence. We met them mainly in bile in which the depressing effect came out only when administered in still larger doses. As a rule, when moderate doses of bile were sufficient to produce complete paralysis and coma, the addition of strychnine was of no avail or had rather a contrary effect, as we shall see later. In cases, for instance, where the bile produced complete coma and paralysis with a dose of two c.c., we never succeeded in overcoming completely the effect by the addition of strychnine, although even in these instances some degree of neutralization was at times noticed. The following experiment illustrates this effect:

Group III.—Bile from the dilated common duct of a rabbit ligated three days before.

Frog A, injected 0.6 c.c. bile; complete coma and paralysis in one hour.
Frog A′, injected 0.6 c.c. bile + 0.005 mgr. strychnine nitrate; some coma, increased reflexes, condition decidedly better than A.
Frog B, injected 1 c.c. bile; after one hour coma and paralysis, but alive.
Frog B′, injected 1 c.c. bile + 0.005 mgr. strychnine nitrate; coma and paralysis very soon; dead at the end of an hour.

Here in A′ the addition of a very small dose of strychnine was apparently instrumental in reducing the toxic effect of the depressing bile. (The effect in B′ will be discussed later.)

The last two series of experiments have brought out two new important facts: 1. That rabbit's bile surely contains a tetanic element. 2. That the depressing and the tetanic elements within the bile are antagonistic to one another and are capable at least to a degree of mutual neutralization. Regarding the mutual relations of the antagonistic factors as they actually exist within the bile we have so far learned the following things:

In the bile of the gall bladder and the ligated common duct the depressing element is apparently the chief factor, the bile producing only coma and paralysis; it is toxic in comparatively small doses. The presence of the tetanic element can be detected only by the addition of an ineffective dose of strychnine and then

not in a very pronounced manner. Perfect neutralization is impossible.

The bile obtained from a biliary fistula of the common duct in general is far less toxic than that from the gall bladder. Rabbits show individual variations. The bile in some is already toxic in the comparatively moderate doses of two c.c. and less. The depressing element is predominant, the effects produced being coma and paralysis. This bile it is impossible to neutralize by the addition of strychnine. However, the doses which are not yet toxic permit without difficulty the bringing out of the tetanic effects by the addition of an otherwise ineffective dose of strychnine.

In still other animals the depressing element seems to be absent, at least it was so in doses up to ten c.c. This bile alone will produce a tetanus readily but only when given in the large doses of five c.c. or more. The addition of an ineffective dose of strychnine will bring out a tetanus with a somewhat smaller dose, but not with doses smaller than two c.c. In this bile, apparently, the tetanic element is also present in small quantities.

Finally, the bile of some rabbits produces hyperæsthesia in doses of two or three c.c. and coma and paralysis in larger doses. The addition of strychnine to comparatively small doses of this bile (one to three c.c.) will produce a tetanus. The addition of strychnine will bring out a tetanus also with doses of this bile just sufficient to bring out (without strychnine) coma and paralysis. When the dose of bile is distinctly above this minimum, coma and paralysis will be the result even if strychnine is added. Both elements are present and struggling against each other. In larger doses the depressing element is the victor, in smaller doses the tetanic element is capable of manifesting its presence, but produces only moderate symptoms. In doses near the border line the addition of strychnine is capable of turning the balance in favor of the tetanic element.

These various observations seem to demonstrate the following:

1. That for the depressing element there is a definitely effective minimum which, when present in a dose of any bile, overcomes

nearly completely the antagonistic tetanic element, no matter in what strength the latter is present.

2. That bordering on this effective minimum there is another minimum strength of the depressing element which, when present alone, or nearly so, can produce coma and paralysis; but this effect can be overcome by the antagonistic tetanic element when simultaneously present in the bile in a strength sufficiently above its minimum.

3. That this last minimum of the depressing element or a strength weaker but near to it, when mixed with a minimum effective dose of the tetanic element might lead through mutual neutralizations to normal effects.

4. That the tetanic element when alone is efficient in a much smaller strength than the strength which is required for the depressing element to be efficient; in other words: the effective minimum for the depressing element lies higher than that for the tetanic element.

With these relations of the antagonistic elements in mind, we can easily understand why some bile causes only depression, other bile only hyperæsthesia and convulsions, and still other bile produces hyperæsthesia in smaller and coma and paralysis in larger doses.

Remembering these considerations it may be further conceived that bile which would cause hyperæsthesia or even tetanus in small doses, might produce normal conditions with larger doses, and coma and paralysis with still larger doses. Such apparently paradoxical conditions would occur when both antagonistic elements were present in the bile simply in relatively minute strengths. The gradual increase of the dose of the bile would first bring the tetanic element up to its effective minimum while the depressing element would still be far below its effective strength, and the result would be hyperæsthesia or convulsions. A further increase in the dose of the bile would bring the depressing element up to a strength approaching its effective minimum, or just sufficient to neutralize the tetanic element even in its present strength; the result would be an apparently normal condition. Finally, a further increase of the dose of

bile would bring the depressing element up to the effective strength which is capable of completely overcoming the tetanic element in any strength; hence the coma and paralysis.

These paradoxical effects have actually been present in a few instances, especially in the experiments with additions of subminimum doses of strychnine. The following protocols are illustrations.

Group IV. Frog 1, injected 1.5 c.c. of bile from nephrectomized animals at 11.20 A.M. 2 P.M., frog normal. 6 P.M., rigidity of legs on handling; next day normal.

Frog 2, injected 3 c.c. of same bile at 11.10 A.M. 2 P.M., normal. 2.30 P.M., somewhat "stupid," otherwise normal, no increase of reflexes; next day normal.

Frog 3, injected 5 c.c. of same bile at 11 A.M. 2 P.M., coma and paralysis. 6.30 P.M., dead.

In this experiment Frog 2 had no rigidity or increased reflexes and was in better shape than Frog 1, while Frog 3 had coma and paralysis.

Group V. Frog 1, injected 1.5 c.c. bile from nephrectomized rabbit and immediately after injected 0.01 mgr. strychnine at 11.20 A.M. 2 P.M., violent tetanus, passed into coma. 6.30 P.M., found dead.

Frog 2, injected 3 c.c. of same bile, then 0.01 mgr. strychnine at 11.30 A.M. 2 P.M., increased reflexes. 6.30 P.M., increased reflexes, somewhat comatose (?). Next day normal.

Frog 3, injected 5 c.c. of same bile, then 0.01 mgr. strychnine at 11 A.M. 2 P.M., coma and paralysis. 6 P.M., dead.

Frog 2 with the larger dose of bile had neither tetanus nor coma and survived, while Frog 1 with the smaller dose showed both and died. The rapid development of coma after a violent tetanus we will discuss later.

Group VI. Frog A, injected normal bile 2 c.c. at 10.30 A.M. At 2 P.M., reflexes increased. 6 P.M., normal.

Frog A′, injected normal bile 2 c.c., then 0.01 mgr. strychnine at 10.30 A.M. At 2 P.M., marked increase of reflexes, rigidity on handling; next day normal.

Frog B, injected normal bile 3 c.c. at 10.30 A.M., at 2 P.M., stupor and paresis; at 6 P.M., coma and paralysis; next day dead.

Frog B′, injected normal bile 3 c.c., then 0.01 mgr. strychnine at 10.30 A.M. 2 P.M., normal; 6 P.M., normal; remains normal next day.

Frog C, injected normal bile 5 c.c at 11 A.M.; 2 P.M., coma and paralysis; 6 P.M., dead.

Frog C′ injected normal bile 5 c.c., then injected 0.01 mgr. strychnine at 11 A.M.; 2 P.M., coma and paralysis; 6 P.M., dead.

In this experiment the addition of strychnine to two cubic centimeters of bile brought rigidity, while the control animal remained normal; and the addition of the same amount of strychnine to three cubic centimeters of bile brought about normal conditions while the control died from coma and paralysis. Finally, the addition of the same amount of strychnine to five cubic centimeters of bile did not reduce the paralyzing effect of a large dose of bile, and there was practically no difference between the frog given strychnine and the control. The addition of strychnine brought out then first tetanic symptoms; when the quantity of bile and with it the strength of the depressing element were increased, the antagonistic elements were just sufficient to neutralize one another. When, however, the depressing element was increased considerably, it not only neutralized all tetanic effects but had a sufficient balance left to produce paralysis and coma.

These striking results were, however, rarely met with. They would seem to depend upon so many circumstances more or less uncontrollable as to make their intentional or even accidental production infrequent. But instances of mutual neutralization of less striking character were much more frequently produced.

The addition of ineffective doses of strychnine to the bile of nephrectomized rabbits brought out instructive observations. From the results in Series IV, we had reasons to surmise that a certain amount of a tetanic element of metabolic origin, which is normally eliminated through the urine, passed in nephrectomized rabbits through the bile. We compared in a number of experiments bile from nephrectomized rabbits with that of normal animals as to the effects of the addition of strychnine. In the majority of experiments the difference was very strikingly in favor of the bile from nephrectomized rabbits being rendered more tetanic than normal bile by this addition. Some of the experiments follow:

Group VII. Frog A, injected at 3 P.M. 3 c.c. of normal bile, and then at 3.25 P.M. 0.01 mgr. strychnine. No signs of tetanic effect; paralysis; dead next day.

Frog A′, injected at 2.30 P.M. 3 c.c. bile from nephrectomized rabbit, and at

3.20 0.01 mgr. strychnine. At 3.40 legs rigid, later tetanus on handling; normal next day.

Frog B, injected 5 c.c. normal bile at 2.40 P.M, and at 3.25 added 0.01 mgr. strychnine. No signs of a tetanic influence. Coma, paralysis, and death.

Frog B′, injected 5 c.c. of bile from nephrectomized rabbit at 2.40 and at 3.25 added 0.01 mgr. strychnine. At 4.25, distinct rigidity on handling, and at 5, distinct tetanic attacks. Normal next day.

Group VIII. Frog A, injected at 12.30 P.M. 5 c.c. normal bile, and immediately after 0.01 mgr. strychnine; 2.30, coma and paralysis; dead next day.

Frog A′, injected at 12.30 P.M. 5 c.c. of bile from nephrectomized rabbit, and immediately after 0.01 mgr. strychnine. 2.30, increased reflexes and rigidity of extremities; no coma; next day reflexes still increased, and on hitting table, mild tetanus. Survived.

In these and in some additional experiments it was shown that the addition of ineffective doses of strychnine to comparatively large doses of bile of nephrectomized rabbits produced tetanic symptoms, while the same doses of strychnine and normal bile produced coma and paralysis. In other words, nephrectomy apparently increased the tetanic action of the bile.

We have up to now dwelt chiefly on our observations upon the increase of the tetanizing effects of bile by the addition of ineffective doses of strychnine. We have now to state that under certain conditions the addition of strychnine favors the appearance of the depressing effect of bile. Indeed among the experiments described above were some in which this action of strychnine in favoring the appearance of coma and hastening the death of the frogs was shown. This effect has been noted by us in the case of the bile from a large number of rabbits as well as other animals. These observations may be summed up as follows: When in employing doses of bile which, when injected alone, never produced any degree of coma and paralysis, there was added strychnine, no matter whether in small or large doses, it never favored the appearance of the depressing phenomena; if, however, the bile caused alone the appearance of coma and paralysis quite readily, and the dose employed was one producing sooner or later some degree of coma and paralysis, then the additional injection of strychnine frequently hastened the onset and increased the rapidity of development of the depressing effects In some cases the sudden onset of coma was preceded by very

brief and rarely by very violent tetanus. The tetanus was usually so brief that it may in other instances have frequently been overlooked. The larger the added amount of strychnine, the more quickly the tetanus and the sooner complete coma and paralysis would set in.

In cases where coma and paralysis are brought on by bile alone, the development of the depressing symptoms takes place gradually and slowly, a fact especially true of doses not too large. The usual course is as follows: After the animal recovers its activity immediately after receiving bile it gradually becomes abnormally quiet and remains sitting in one place with the head gradually sinking down to the table. At this stage handling arouses it for a while and it jumps away in a normal manner, but as soon as left alone it becomes again droopy. Soon it offers little resistance, very often it does not jump away, and when it does jump it does so clumsily for short distances. When turned on its back it makes only feeble attempts to turn on its stomach. However, once in a while it succeeds, and when again on its stomach draws its legs near the body, the head and stomach now resting on the table. Gradually the limbs become limp and in the attempts to move they get away from the body; their tonicity is lost and they look œdematous, after which the animal soon appears to be perfectly paralyzed. Occasional sudden, feeble twitchings of some parts of the body occur, the respiration becomes feebler and slower, the heart-beats become diminished. At this stage the pupils are generally constricted. The frog can remain in this condition for many hours before all signs of life disappear. Between the injection and the state of complete coma and paralysis many hours often elapse.

The course is entirely different when in addition to an effective dose of bile a dose of strychnine, especially one approaching the effective minimum, is given. On being put under the glass bell the animal becomes very lively, keeps its head high, and is continually moving around; it is always near the glass wall looking for opportunities to escape. On hitting the table suddenly or handling the animal, and sometimes even without any visible cause, the frog is seized with a tetanic convulsion and a few

seconds later is lying stretched out as nearly paralyzed as a frog in advanced stage of depression from bile alone. A blow on the table may still bring on brief responses of feeble stretching of the extremities, but in a short time there is no visible difference between the paralysis of the strychnine frog and the paralysis brought on simply by the bile.

As to the cause of this paradoxical effect of strychnine, there are several possible explanations. We may think in the first place of the possibility that strychnine, containing as is well-known a certain depressing element, manifests this action after the tetanus is over, and hence we might assume that it is this depressing effect added to that of the bile which hastens the onset of complete depression. It is probable also that the sudden onset of coma is always introduced, as we have already mentioned above, by a brief tetanus which on account of its slightness is frequently overlooked. We may then assume further that after the convulsive explosion, the resistance of the tetanic element becoming momentarily exhausted, the depressing phenomena appear suddenly. Finally, the normal interval between the injection of the bile plus the strychnine and the outbreak of acute coma is upheld by a very unstable equilibrium between the two antagonistic elements. The sudden outbreak of the tetanic condition disturbs this equilibrium, with the result that henceforth the stronger factor, in this instance the depressing element, dominates the field. It is perhaps possible that the acute outbreak is brought about by a combination of all the factors mentioned in the three possibilities.

We have stated that after the outbreak of the coma and paralysis the frogs remained in a state of depression until death. This is the usual outcome. In a few instances, however, the course up to the acute onset of the coma was the same as in all the other experiments. The further part of the course, however, was strikingly different. The animal would lie apparently in complete coma and paralysis; but a stimulus of any kind—such as touching the frog or striking the table—would bring on a pronounced tetanic attack of short duration which would pass rapidly into complete coma and paralysis. Sometimes these

tetanic attacks would break out apparently spontaneously. This alternation between tetanus on the one hand, and coma and paralysis on the other, would continue for many hours, the condition reminding one in a striking way of the syndrome in human pathology designated "eclampsia."

The quantity of strychnine employed in these cases had to be at least near the minimum effective dose; the quantity of bile, on the other hand, had to be not too much above the minimum dose which when employed alone brought on coma and paralysis. When large doses of depressing bile were injected no such effect could be brought on by the addition of even a large dose of strychnine.

The tetanus occurring in these "eclamptic" frogs was markedly in contrast with the tetanus brought on by bile alone. Each single tetanic attack in the latter cases lasted a long time, and after passing off left behind a marked tonicity of the muscles, giving to the animal a somewhat "graceful" appearance. Each tetanus in the "eclamptic" state was very brief and passed abruptly over into perfect relaxation, which gave the animals an "œdematous" appearance.

Finally, we have to add that, when after the injection of bile coma and paralysis were already completely developed, the injection of strychnine even in doses of 0.04 or 0.05 milligramme hardly ever exerted any influence.

The paralysis which bile produces is in the first place of central origin. This fact has been demonstrated by Rywosch and others by direct experiment. We repeated one of the experiments. In a completely paralyzed animal which received the bile injection, electrical stimulation of the cord produced no effect upon the muscles of the trunk or the extremities; while stimulation of the sciatic nerve produced quite active contractions of the extremities.

That bile also affects nerve and muscle directly was shown long ago by many observers. In our experiments we frequently had occasion to observe this effect in the following manner: In order that the large quantities of bile which we introduced should not escape again, we injected the lymph-sacs by passing

the needle through the muscles of the thigh, the muscles thus working as a valve. Now these legs, and especially the thigh, were often paralyzed long before there was any sign of paralysis in the rest of the frog. In some instances the frog would respond with a tetanus in which even the lower part of the paralyzed extremity would take part. The paralysis of the thigh in those cases was caused undoubtedly by the bile coming in direct contact with the muscles of the thigh. This would mean that even that bile which failed to produce paralysis through the central nervous system might still reduce the irritability of the muscles by coming in direct contact with them.

The reduction of the irritability and conductivity of nerves and muscles by bile had probably some share in the advanced stages, and possibly also in the earlier stages, of paralysis. Of the latter we had to think especially when the injection of bile was given into the lymph-sacs, while the animal remained in its normal position, as was the rule. It might thus happen that some bile reached the sciatic plexus or nerves and caused a premature local paralysis of the posterior extremities. Indeed we met with these examples in cases in which ineffective doses of strychnine in addition to bile were employed, and in which the front legs became rigid while the posterior extremities were paretic or paralytic. In a few cases, therefore, immediately after injecting the bile into the lymph-sacs, we have suspended the frogs by the legs, causing the bile to accumulate preferably in the anterior parts of the body. In these experiments it seemed, indeed, that the addition of strychnine brought on rigidity of the posterior extremities very readily and distinctly, while the animal seemed otherwise to be in a state of stupor.

We should mention further that of the rabbits' bile which we have employed some was received sterile, some was sterilized, and some was exposed to boiling for half an hour before it was used. A few of the experiments with the last bile made the impression that boiling reduces the activity of the depressing element and favors the appearance of tetanus. However, the analysis of all the experiments does not positively justify such a claim. It may be stated with safety that boiling does not affect

in a pronounced manner either of the biliary elements described. But in a number of comparative experiments it was shown that boiling reduces the toxicity of the bile as a whole. The animals which received boiled bile survived pronounced attacks of tetanus as well as some conditions of coma and paralysis, whereas all the control animals with the same amount of unboiled bile, which otherwise produced the same symptoms, died a day or two after the injections. Our experiments were not extensive enough in this regard to permit a more definite statement.

The numerous facts communicated in the foregoing pages were derived, as has frequently been stated, from the study of rabbits' bile. We also stated that the toxicity of the bile of other animals and of bile salts was studied. These latter studies were not as full as the other and were restricted to ascertaining a few essential facts. The results of these studies will now be reported briefly.

Guinea-Pigs' Bile.—The bile of only three guinea-pigs obtained from a cannula fastened either in the common bile duct or in the gall bladder was at hand. The quantity yielded was small and our observations therefore are limited to twelve frogs.

Two or even three c.c. of fistula bile caused no toxic effects of any kind. One c.c. of stagnated bile from the gall bladder also had no effect. The addition of 0.01 mgr. strychnine did not bring on hyperæsthesia. The addition of 0.02 mgr. strychnine brought on, after many hours, distinct rigidity, while the control animal remained normal.

These few results are recorded without attaching much importance to them. The observations were too few and too little varied to permit positive conclusions. It may be said, however, that, few as they were, they were more numerous than some from which certain writers drew their conclusions regarding the nature of the toxicity of bile.

From the fact that even three cubic centimeters were without toxic effect we were at first inclined to the general conclusion that guinea-pigs' bile is less toxic than rabbits', but this notion was dissipated by the experiments with rabbits' bile in which the toxic dose was definitely greater than this amount. It will take a more extensive study to decide whether guinea-pig's bile is indeed less toxic than rabbit's.

Dogs' Bile.—Our experience with dogs' bile is also limited. The few experiments made, gave, however, some definite results in accord with those obtained with rabbits' bile.

Group IX. Frog 1, control, injected 0.01 mgr. strychnine at 3.50 P.M. No effect.

Frog 2, injected 0.5 c.c. boiled dogs' bile (from fistula) at 3.50 P.M. No effect.

Frog 3, injected 0.5 c.c. boiled dogs' bile followed by an injection of 0.01 mgr. strychnine at 3.50 P.M. At 4.30, exaggerated reflexes followed soon by typical tetanus.

In this experiment 0.5 of the bile had no effect, but the addition of an ineffective dose of strychnine brought out a typical tetanus. That means that 0.5 of the dogs' bile contained a tetanic element large enough to convert a subminimum dose of strychnine into a toxic one.

Group X. Frog 1, weight, 36 grammes; injected 1 c.c. of dog's bile at 10.15 A.M. At 12 M., complete coma and paralysis.

Frog 2, weight 42 grammes; injected 1 c.c. of dogs' bile and immediately afterwards 0.01 mgr. strychnine at 10.15 A.M. At 10.50, complete coma and paresis. 12 M., dead.

One c.c. of dogs' bile caused complete coma and paralysis in about two hours; the addition of strychnine hastened the onset of the depressive phenomena and death, an experience similar to that frequently seen in case of the rabbits as described.

Hyperæsthesia and convulsions did not occur after injections of dogs' bile without the addition of strychnine. Still our experience with this bile was too small to permit the drawing of any conclusion.

Ox Bile.—Ox bile as it was obtained from the slaughter-house and inspissated bile dissolved in various concentrations were employed. The bile frequently was filtered and sterilized before using. Our experience with this bile was quite extensive, and hence the few results reported were conclusively established. It is desirable to keep in mind when comparing the effects of ox and rabbit bile that all of the former was obtained from the gall bladder.

Ox bile is very toxic; 0.3 c.c. will, as a rule, cause sooner or later toxic symptoms and death. The former are nearly

exclusively depressive, namely: coma and paralysis. In all our experiments we came across tetanus once only. This frog received 0.3 c.c. of bile; first developed coma; but the next day the coma was interrupted by tetanic attacks—in other words, an eclamptic state supervened. Smaller doses never gave even hyperæsthesia. Since this effect is similar to that obtained with rabbits' bile taken from the gall bladder or from the dilated common duct, stagnated bile is apparently very toxic, the depressing element being the controlling factor in it. We observed, however, numerous proofs of the existence of a tetanic element even in this depressing bile.

Group XI. Frog A, received 1 c.c. of a 10 per cent. solution of ox bile. Condition good; no effect.

Frog A', received 1 c.c. of a 10 per cent. ox bile followed by 0.005 mgr. strychnine at 4 P.M. At 6 P.M., increased reflexes.

Frog B, received 1.25 c.c. 10 per cent. ox bile; condition remained good.

Frog B', received 1.25 c.c. 10 per cent. ox bile followed by 0.005 mgr. strychnine at 4 P.M. At 5 P.M., violent tetanus.

In this series neither the doses of bile employed alone nor strychnine alone produced any effect. When, however, both were given, the effects produced were hyperæsthesia and sometimes tetanus.

Moreover, in some instances when the toxic dose of bile was on the border line and tended sooner or later to bring on depressing symptoms followed by death, the addition of strychnine still brought out tetanus. Of this class of effects the next experiments form examples.

Group XII. Frog 1, injected 0 25 c.c. ox bile at 11.30 A.M. At 3 P.M., marked stupor. Next day, dead.

Frog 2, 0.25 c.c. ox bile, followed by 0.01 mgr. strychnine injected at 11.30 A.M. At 3 P.M., slight stupor. Next day, distinct tetanus.*

On the other hand, we observed many instances in which a dose of 0.01 milligramme strychnine or more, when superadded to a distinctly toxic dose of ox bile, hastened the onset of the depres-

* Such effects remind one of the action of certain doses of morphine upon frogs. On the day of injection there is either no effect or a moderate stupor, while a day or two later a distinctly tetanic condition develops.

sive symptoms and death. In some of these the coma, which was brought on acutely by the addition of strychnine, was not of a continuous, but rather of an "eclamptic" character.

There were some slight variations in the toxicity of the bile obtained at different times. Whether this variation was due to individual differences in the animals we cannot state, since we are not sure that each portion of bile was obtained from a single animal.

Ox bile is therefore highly toxic in small doses and exceeds in toxicity gall bladder bile of rabbits; but like the latter its effect is essentially depressive. The addition of ineffective doses of strychnine shows, however, that ox bile contains an effective tetanic element, the manifestation of which is suppressed by the superior antagonistic effect of the depressing agents. In other words, by the addition of strychnine to ox bile all the phenomena observed in the study of bile from the common duct of rabbits plus strychnine were produced.

Bile Salts.—Glycocholate and taurocholate of sodium were studied separately. According to Rywosch,[19] the fatal dose of sodium taurocholate for frogs weighing from 40 to 50 grammes is about 60 to 70 milligrammes, and of sodium glycocholate about 100 milligrammes. Judging by his protocols Rywosch had in mind doses which produced coma and complete paralysis in less than half an hour and death in less than two hours. In most instances the frogs which we used weighed between 30 and 35 grammes. We considered the bile salts toxic when a given dose produced pronounced toxic symptoms within a few hours. These experiments may be summed up briefly as follows:

A few frogs responded to injections of 20 milligrammes of bile salts, and more responded with distinctly toxic symptoms and a fatal result on the day of injection, or within the next few days to 30 milligrammes. In some instances even 40 milligrammes were without positive effect, and exceptionally some effect was obtained with 15 milligrammes. Fifty milligrammes or more were invariably highly toxic.

The toxic symptoms were stupor and complete coma, paresis,

[19] Rywosch, l. c.

and complete paralysis. Beginning with small quantities and gradually increasing the doses of the bile salts until definite and fatal symptoms set in, we came across only one instance for each salt of a tetanic effect of doubtful character. The exceptions were as follows: With a dose of sodium glycocholate of 40 milligrammes, there were no perceptible symptoms on the same day, but on the next day some tonic rigidity seemed to be present. In another instance about half an hour after injection of 25 milligrammes of sodium taurocholate coma developed, and while handling the frog a slight rigidity set in which soon passed over into complete paralysis.

These two doubtful instances of temporary rigidity were the only signs of the presence of a tetanizing influence among the experiments with the bile salts. There was in this regard no perceptible difference between the effects of sodium taurocholate and glycocholate. It should also be added that in our experiments no difference in the toxicity of the two salts was noticed, a point of difference with Rywosch.

The experiments with the addition of strychnine did not bring out any striking results which favor the notion of the presence of a tetanic element in the salts. Less than 0.01 milligramme had not the slightest effect. Of many experiments with additions of 0.01 milligramme of strychnine to the dose of sodium glycocholate, in one only was slight hyperæsthesia noted. This amount of strychnine when added to sodium taurocholate (10 mgr. salt + 0.01 mgr. strychnine, mixed) produced in one case, after 2½ hours, a passing tetanus, and in another case (30 mgr. + 0.01 mgr.) a slight tetanus. In the many other experiments with the taurocholate (strychnine 0.01 and 0.015) there was no effect except perhaps a slight hyperæsthesia in one or two instances. The addition of 0.02 milligramme of strychnine to sodium glycocholate brought out tetanus in two experiments, while the control with strychnine alone gave only hyperæsthesia; in other instances there were only increased reflexes. We had the same experience with sodium taurocholate.

In a few instances the addition of a larger dose of strychnine hastened the onset of coma. Two of the above-mentioned at-

tacks of tetanus were the precursors of coma and paralysis which continued until death, except in two examples where the course was of a typically "eclamptic" character.

The general conclusions which our experiments with bile salts permit us to draw are that the toxic effects of the salts upon the general nervous system are essentially depressing in character. The salts probably also contain the tetanic element, but in very small amount, hence the addition of strychnine is distinctly less effective than even with bile which comes from the gall bladder.

In reviewing our results we shall trace our steps backwards. The investigation, which we began in the attempt to solve our original problem, led us to the question of the actual nature of the toxic action of bile. As an answer to this question we obtained a number of facts the signification of which we wish to present.

It is now generally accepted that the toxic effects of bile are only of a depressing character. In our studies we found this to be true for the bile obtained from the gall bladder of the rabbit and ox, at least as far as the actual effect is concerned. In a few instances, in which we made experiments, this fact held good for the gall-bladder bile of dogs and guinea-pigs. Sodium taurocholate and glycocholate also proved to have a depressive action.

It was, however, different with bile obtained from a fistula of the common duct of rabbits. The bile of certain rabbits produced hyperæsthesia and true tetanus, but never coma and paralysis. The bile of other rabbits produced marked hyperæsthesia with small doses, and coma and paralysis with large ones. Only in a few experiments did this bile cause coma and paralysis and no increase of excitability. Hence we have positive and direct proof that normal bile from many rabbits possesses an exciting element capable of producing clonic and tonic convulsions in frogs.

We found that small doses of bile of any kind could cause hyperæsthesia and tetanus when supported by small doses of strychnine. The doses of the bile and strychnine which come

into play in these experiments were below that which separately produced symptoms. This could only mean that bile contains an element which like strychnine can produce increased excitability, and which can supplement it. We designate this hypothetical agent in bile for sake of brevity as the tetanic element. By the same method we have demonstrated the existence of a tetanic element in the bile of the common duct of rabbits which alone gave only depressing symptoms, and in the gall-bladder bile of dog, ox, and rabbit. The evidence obtained for the presence of the tetanic element in bile salts was not conclusive. If present at all, it apparently occurs in smaller quantity than in the whole bile.

We may, therefore, claim from our experiments that we have established the fact that bile from the common duct of some animals, at least, contains a tetanic element which may manifest its presence without the aid of strychnine; and, in some instances, would appear to be the sole toxic agent present.

Gall-bladder and stagnant bile of all animals produce depressing symptoms, although they surely contain a tetanic element.

From Series II and III it would appear that bile which is obtained soon after the preparation of a fistula is apt to cause depressive symptoms chiefly.

In some animals indeed all the bile from the common duct causes in comparatively small doses only coma and paralysis. It should be added that the flow of bile in these animals was slow, and the quantity collected was small as compared with that obtained from animals the bile of which produced hyperæsthesia and convulsions. All these facts considered, it may be stated that stagnant bile produces depressing symptoms.

Our experiments have demonstrated not only that all kinds of bile contain the tetanic element, but also that this and the depressing element are present simultaneously. In stagnant bile, though it surely contains both elements, it is, apparently, the depressing element essentially which manifests its effects. What are the mutual relations of these two toxic agents? From our experiments with the addition of strychnine we may answer this very difficult question as follows:

1. That the depressing effect of large doses of bile cannot be reduced or visibly modified by the addition of any dose of strychnine.

2. That the depressing effect of doses of bile, in quantities near the toxic minimum, can be promptly modified by a subminimum dose of strychnine: (a) The effect can be neutralized or annihilated, whence a normal condition results; (b) the effect can be overcome to such an extent as to bring out the opposite or tetanic effect.

3. That by an increase of the depressing element the positively tetanic effect of strychnine plus the tetanic element in the bile can (a) be neutralized, i. e., reduced to normal, or (b) made to produce coma and paralysis.

These observations lead us to assume in the first place:

That the depressing and tetanic elements are exact antagonists and that their joint effect is that of an algebraic sum.

That, furthermore, when each of the antagonists is simultaneously present in the bile in a distinctly effective minimum, the effect of the depressing element is by far the stronger, so as to conceal any modifying effects of its antagonist.

That the tetanic element attains its minimum effectiveness in a much smaller dose than is required for the effective minimum of the depressing element.

On the basis of these interrelations we can explain the following combinations:

When the depressing element is present in a distinctly effective minimum, the result of an intoxication will always be coma and paralysis.

When the tetanic element is present in a strength which is its minimum or above it, while the depressing element is far beneath its own minimum, the result will be hyperæsthesia and possibly tetanus.

When both elements are present in minute quantities, very small doses of the bile will be harmless. With an increase of dose a stage will first be reached in which the tetanic element will attain its minimum, but reduced somewhat in effect by a low strength of the depressing element, a condition leading to

hyperæsthesia. The next increment of increase of dose will be influenced more by the depressing than by the tetanic element, through which the result may be the production of a normal condition, due to exact mutual neutralization. Any further increase of dose will, of course, lead to supremacy of the depressing element. The middle stage, which depends largely upon exact neutralization, will frequently be missed or overlooked.

The assumption that the minimum for the tetanic element lies much lower than for the depressing element,[20] and the fact that the definite minimum of the depressing element always overpowers the tetanic element, readily explain why in stagnant bile the depressing effect is supreme.

Let it be assumed for the sake of illustration that D (depressive) = 2 T (tetanic), and let it be further assumed that 4 c.c. contains D + 2 T, then 4 c.c. will give coma and 2 c.c. ($\frac{1}{2}$ D + T) will give tetanus. When the stagnant bile loses half of its water without losing its toxic elements, then 2 c.c. of this bile will contain D + 2 T, with the result that now 2 c.c. of this bile will cause coma and paralysis instead of tetanus.

In all of these considerations we have however to remember that in the algebraic sum of both antagonists, no matter how small one of the factors may be, none of the effect is actually lost, i. e., if the result is coma and paralysis in some degree, we have to think were it not for the simultaneous presence of the tetanic element the degree of depression would have been higher; and vice versa.

The conditions and relations which we have just described hold good for stable relations only, i. e., when both antagonistic elements exist in such firm relations to one another as to produce a state of equilibrium. When these mutual relations are disturbed, as would for instance happen when one or the other element was suddenly superadded in a certain strength, the outcome would be a struggle for supremacy, which might continue or be early decided for the one side or the other, whence the equilibrium would be broken. We have seen this

[20] An analogy is found in the minimum toxic dose of strychnine (tetanic), which is 10 times lower than the minimum of morphine (depressive).

happen in our experiments where the addition of an effective dose of strychnine to an effective dose of bile brought out a very short tetanic effect, which immediately gave way to a premature onset of deep coma and paralysis. The depressive phenomena were either continuous till premature death, or an alternating change between coma and convulsion, or a state of "eclampsia" occurred.

Finally, we have to point out that in bile salts the tetanic element is apparently present in less amount than in the whole bile. This fact does not agree well with the now generally accepted theory that the entire toxicity of bile resides in the bile salts. An assumption that the tetanic element is intimately associated with some other portion of the bile, not yet isolated in purity, would seem more in harmony with the facts; in which case it might be thought that only a fraction of this element clings to the bile salts. It should also be recalled that in our experiments the tetanic element was not destroyed by prolonged boiling of the bile. Still we do not wish to draw final conclusions from our series of experiments with bile salts.

The results of the series of experiments which we have just analyzed demonstrated that bile from the common duct of normal animals does contain a tetanic element and produces occasionally convulsions, and they also demonstrated that the addition to this bile of a tetanic factor of any source will increase the tetanizing efficiency of that bile. We shall now examine in the light of the last experience the results which we obtained in the first few series of experiments.

In Series IV we found that the bile from the common duct of nephrectomized rabbits showed a tetanic character more frequently than the bile of normal animals in Series V, and much more so than the bile of the ante-strychnine period in Series II (nephrectomized rabbits). We can now safely interpret this observation on the basis of the assumption which we previously suggested, namely, that normally a tetanic agent of catabolic origin is being eliminated in some degree through the urine.

After nephrectomy this tetanic agent finds its way, in part or

wholly, into the bile and thus increases the efficiency of the tetanic agent normally present in this fluid.

This interpretation is also in harmony with the observation that the addition of strychnine to bile from nephrectomized rabbits increases the tetanizing effect of this bile more markedly than bile of normal rabbits.

But as the bile of the ante-strychnine period in Series II is, as we stated above, more or less stagnant, the prevalence of depressive symptoms of intoxication in spite of the added tetanic element is easily understood.

The bile of the post-strychnine period of Series II appeared to be more tetanic than that of any other series. We assume that in this series some at least of the strychnine was eliminated through the bile. Hence the tetanic agents in this bile were of three kinds: the tetanic element of the bile itself, the strychnine, and the tetanic element ordinarily eliminated through the urine. Through the united effort of all the three agents, the effects of the antagonistic depressing element are considerably overcome; whence the considerable increase of the exciting effects of the bile of this series.

The same reasoning applies to the bile of at least two normal animals of Series III which received strychnine. An instructive incident is the observation in these cases that the urine of these animals had nearly no hyperæsthetic effect, while in normal animals which receive strychnine the urine, as a rule, contains so much strychnine as to produce tetanus. Our explanation of the underlying conditions is as follows: Normally strychnine is eliminated through the bile into the intestines, but only to be reabsorbed and to be gradually and finally eliminated through the urine. However, as the bile is continually being discharged outside of the body through a fistula from the common duct, there is less chance for the strychnine to get into the urine.

In the first series of nephrectomized rabbits with ligated common duct the strychnine had diminished avenues of escape from the body, whence the proportionately more frequent occurrence of tetanus in these animals than in animals which were nephrectomized and then received subminimum doses of strychnine.

Furthermore, it is possible that in some cases strychnine accumulated in the gall bladder, the biological test failing to demonstrate its presence on account of the overpowering effect of the depressive element in this stagnant bile.

RESUMÉ.

The preceding investigation can be summarized as follows:

That, contrary to the prevailing opinion, bile contains a tetanic element, or an agent which causes increase of excitability of the nervous system.

That bile from the common duct of many rabbits causes marked hyperæsthesia and tetanic attacks when injected into frogs.

That stagnant bile (as in the gall bladder, etc.) invariably produces coma and paralysis.

That the depressive and exciting elements of the bile are mutual antagonists, the total effect of both, when simultaneously present, being that of an algebraic sum.

That the depressive element when present in a highly effective dose is by far the stronger of the two; while, on the other hand, the tetanic element becomes effective apparently in a dose far below that which constitutes the minimum for the depressive element.

That owing to these peculiar characteristics of the two antagonistic toxic elements, the mixture of both within the bile can be so adjusted as to produce any of the many possible degrees of irritability lying within the range of tetanus at one end and paralysis at the other, and including a neutral state presenting apparently a normal or tranquil condition.

That bile salts seem to contain the tetanic element in a distinctly less amount than the whole bile.

That nephrectomy increases the toxicity of bile in the direction of excitation.

That the effect of nephrectomy can be imitated by subcutaneous injections of strychnine.

That the bile of nephrectomized rabbits which received

strychnine produces hyperæsthesia and convulsions more readily than any other bile.

That, finally, by injecting a toxic dose of strychnine into a frog soon after it has received a distinctly depressing dose of bile, an "eclamptic" state can in some cases be produced.

THE CONSTITUENT OF THE BILE CAUSING PANCREATITIS AND THE EFFECT OF COLLOIDS UPON ITS ACTION.

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The origin of many cases of acute inflammation of the pancreas in a diversion of the biliary secretion from the intestine into the pancreatic duct has been established by Opie's [1] observation and experiments. His experiments did not, however, take into account the nature of the biliary constituent which excites the inflammatory and other changes, neither did they undertake to discover whether varying effects were ever produced by samples of bile of different composition. While Opie's discovery threw much light upon acute hæmorrhagic pancreatitis it did not illuminate particularly the less acute or the chronic forms of pancreatic disease. It is noteworthy that the association of cholelithiasis with pancreatic disease [2] is more frequent than the mere acute affections of the gland, and the coincidence of cholelithiasis with sclerosis of the pancreas is so common as to arouse a suspicion of a possible pathological relationship of these conditions. The experiments which form the subject of the present communication are believed to show, first, the nature of the constituent of bile which acts injuriously upon the pancreatic tissue, and, second, the mechanism by which the injurious action of this constituent is modified and controlled and hence rendered capable of bringing about widely diverse effects. The experiments made upon the dog are believed not to be without application to similar pathological conditions found in man.

The bile contains two sets of constituents of highly different chemical composition: crystalline principles, and colloids. The

[1] "The Etiology of Acute Hæmorrhagic Pancreatitis." *Bull. of the Johns Hopkins Hospital*, 1901, xii, 182.

[2] Opie, *Diseases of the Pancreas*, 1903.

former consist chiefly of the biliary salts and coloring matter, the latter of nucleoalbumin. The biliary salts are known to act injuriously upon cells, for which a measure may be found in the dissolving effects upon red-blood corpuscles[3]; while no such direct cellucidal property is known either for the biliary coloring matter or the mucin.

Bile is, however, a secretion of much complexity and hence it is possible that it contains uniformly, or at times, still other substances of less defined nature which may act injuriously upon cells. The experiments to be related would seem to make unnecessary the assumption in bile of any hypothetically injurious substance upon which depends the power to set up inflammation of the pancreas.

The first experiments were made with a sample of sodium taurocholate as representing the main bile salt of the dog's bile. The specimen was brownish in color and gave on solution a yellowish-brown fluid. The salt was dissolved in a proportion approximating the biliary salt strength of dog's bile, namely, 0.650 gram of the salt in five cubic centimeters of water or saline solution.[4] The injection was made directly into the pancreatic duct as it passed into the intestinal wall. The duct was frequently tied after the injection. The later experiments with bile salts were made with samples of the total salts prepared directly from dog's bile. The method was to treat gall-bladder bile with absolute alcohol in large excess, separating the precipitated mucin and in part the coloring matter. The alcoholic extract was evaporated to dryness on a water bath with animal charcoal. The dried mass was repeatedly extracted with absolute alcohol, and the alcoholic extract after moderate concentration was precipitated with ether. The salts were quickly separated by filtration with suction and dried over sulphuric acid. They were obtained in a white state. The muciginous residue from the first alcoholic extraction was dried and used in certain experiments in which the elements of the bile were recombined.

[3] Rywosch, *Inaugural Dissertation*. Dorpat, 1901.
[4] Shafer, *Text-Book of Physiology*, I, p. 370.

Experiment 1. 3. 15. 05. Brown mongrel. 11 A.M., injected into main pancreatic duct 5 c.c. solution of 0.650 grm. white bile salts in water. Duct tied. Loop of intestine exposed quickly became congested. Pancreas at place of injection became immediately congested, and in 2 or 3 minutes stasis or hæmorrhage occurred. 5 P.M., dog lying in farthest corner of cage; very sick 3. 16. 05., very sick; dead, 2 P.M. Autopsy: No excess of fluid in the peritoneal cavity; serosa, except for fat necroses to be mentioned, smooth and glistening. These fat necroses are typical in appearance and are scattered in the omentum and mesentery. The pancreas, except a small fraction of the duodenal end, shows swelling, hæmorrhage, and necrosis. The hæmorrhagic areas occupy spaces in the pancreatic substance and reach an extent of 2 x 3 cm. Fat necrosis also occurs in the interstitial pancreatic tissue.

Experiment 2. 3. 23. 05. Pug. 10.30 A.M. Same injection as in previous experiment. Almost immediate extreme congestion and hæmorrhage into injected portion of the organ. Dead, 3. 25. 05., during night. Autopsy: Peritoneal cavity contains much bloody fluid; serosa covered with fine, granular exudate. Fat necroses occur in the omentum, mesentery, and fat about operation wound. Adhesions occur between the liver, pancreas, and colon, which on separation disclose a cavity the size of a small orange containing dark bloody and slightly offensive fluid. The walls, composed of pancreatic tissue, are necrotic and covered with fat necroses and hæmorrhages. In other parts the pancreas shows softened areas of varying size (reaching 3 to 4 cm.), which prove to communicate with the larger cavities. The substance of the gland contains large, unsoftened necrotic foci of pancreatic tissue and fat.

The power to set up inflammation of the pancreas of high degree is proven for the bile salts by these experiments. The fatal nature of the inflammation could be further emphasized by citing other experiments, but these should suffice. In one experiment with bile salts death occurred in eight hours, by which time the entire pancreas was swollen and diffusely infiltrated with blood. The fact should be emphasized that besides the typical picture of experimental pancreatitis in the dog the bile salts have produced in one instance (Experiment 2), in the brief space of one and a half days, a condition of hæmorrhagic and gangrenous inflammation of the gland.

The next experiments relate to the action of the alcohol-insoluble or muciginous residue, in the stating of which an account is also given of a third series of experiments, in which the two separated portions of the bile were used after recombination.

Experiment 3. 3. 15. 05. 10 A.M. Brown Skye terrier. 5 c.c. of an aqueous suspension of the alcohol-insoluble residue injected. The suspension was pale brownish-yellow in color and of muciginous consistence. Duct tied.

No discoloration of pancreas except from injection; no congestion. 5 P.M. Dog bright; walks about. No subsequent symptoms. 3. 20. 05. Chloroformed. No lesion of pancreas except, possibly, slight sclerosis from ligation of the duct. No general increase in consistence of the organ.

Experiment 4. 3. 15. 05. White and brown cur. 10.30 A.M. 5 c.c. of the bile-residue suspension of Experiment 3 plus 0.650 grm. bile salts. Duct tied. Immediate discoloration followed by stasis or hæmorrhage of the pancreas in injected area. The duodenal loop of intestine became greatly congested. 5 P.M. Dog appears bright and is up. 3. 20. 05. Dog looks well. Chloroformed. General peritoneal cavity normal in appearance. The pancreas in part is swollen and hæmorrhagic. The duodenal tail is the most normal in appearance, but the tissue immediately adjacent to this is firmer than normally and of a roughened appearance. This pale, firm tissue passes into the acutely swollen part of the gland, which, on section, shows hæmorrhagic and necrotic foci. In this process the entire mid- and splenic-portions are involved.

The absence of all inflammatory power in the alcohol-insoluble residue is brought out by these experiments, which also point to a positive property of this residue, namely, that of reducing in activity the biliary salts. Since this is a property of some importance still another set of experiments will be given.

Experiment 5. 3. 1. 05. Black mongrel. 11 A.M. Injected 5 c.c. of dissolved alcohol-insoluble residue. Duct not tied. Chloroformed, 3. 4. 05. Pancreas appears normal except at the point of separation from the intestinal wall in exposing the duct. A few small necroses in the fat at this place.

Experiment 6. 3. 1. 05 White mongrel. 12 M. 0.650 grm. sodium taurocholate (Merck's) in 5 c.c. aqueous suspension of alcohol-insoluble residue. Injected portion of pancreas became immediately congested, possibly hæmorrhagic. 3. 3. 05. 10 A.M. Dog recovered from operation; looks well. Chloroformed. Peritoneal cavity shows nothing abnormal except a small quantity of bloody fluid in the pelvis. Pancreas, except duodenal end, greatly swollen, and the serous surface covered with bloody exudate. Very few areas of fat necrosis, limited to region about pancreas. On section, the swollen pancreas permits much blood-stained serum to escape. The hæmorrhagic and œdematous areas are somewhat separated, the former being in the parenchyma, the latter in the interstitial tissue chiefly. The duodenal extremity alone has escaped.

These experiments leave no reasonable doubt of the modification by other biliary constituents of the effects upon the pancreas of bile salts. The modification is expressed by, first, the less fatal character, and, second, less acute and destructive nature of the inflammation of the gland. The latter changes, as compared with the severer fatal inflammations, do not differ so much in extent as in intensity, and while hæmorrhage and œdema com-

monly exist marked necrosis is not so prominent. Since, however, the natural full bile is able to cause an acutely fatal inflammation we should also expect the recombined bile to do the same unless the chemical processes have permanently altered the constituents. The next experiment bears on this subject.

Experiment 7. 3. 23. 05. Brown dog. 10.45 A.M. 5 c.c. resuspended bile residue plus 0.650 grm. bile salts injected. The suspension was thinner than previous ones; mixture kept in thermostat at 60° C. for ½ hour. 3. 24. 05. 6 P.M. Dead. Autopsy: The peritoneal cavity congested, and contains excess of fluid. The pancreas is softened, congested, and hæmorrhagic. The section of the gland shows intermingled areas of necrotic parenchyma, hæmorrhages, and fat necrosis; moderate number of peritoneal fat necroses.

It was, of course, desirable to determine the nature of this inhibitive action upon the bile salts. Two possibilities presented themselves: the first, that a directly antagonistic substance exists in the bile which tends to inhibit the salts; the second, that the restraining action is bound up with the physical properties of the medium, since the proteid constituents of the bile give a colloid quality to the mixture.

Experiment 8. 3. 22. 05. Brown mongrel. 11 A.M. 5 c.c. semi-solid mixture, at room temperature, of agar-agar and gelatine was melted and in it was dissolved 0.650 grm. bile salts. Mixture kept at 60° C. for 30 minutes; cooled to 40° C. and injected. Duct not tied. 3. 30. 05. Chloroformed. Dog has lost considerably in weight. Autopsy: General peritoneum normal. Firm adhesions about pancreas, liver, stomach, and intestine On exposing the pancreas it appears shrunken and is firmer and more nodular than normally. Only the duodenal end retains its normal appearance. The main part of the gland is grayish-white in color and infiltrated, here and there, with blood. An area of necrosis the size of a bean and extending to the surface occurs in the mid-duodenal portion. On section, the whole gland, except a superficial sheath or armor of pancreatic tissue below the capsule, is necrotic, grayish, and soft, and gas bubbles in small number slowly disengage themselves from the cut surface. Odor not offensive. On cover slips bacteria are not numerous, but of varied morphology.

That a bland colloidal solution can restrain the activity of the otherwise very highly destructive and certainly fatal bile salts is shown conclusively by this experiment. Several experiments were next made with the whole bile in which the colloid strength was increased by the addition of bland colloidal substances or diminished by diluting the bile with saline solution.

Experiment 9. 11. 13. 05. Two dogs of equal size. 12–1 P.M. Brown mongrel: 2 c.c. bile plus 2 c.c. 5 per cent. agar-agar. Mixture cooled to 40° C. Duct tied. No immediate effect on pancreas. White cur: 2 c.c. bile plus 2 c.c. salt solution. Duct tied. No immediate action on pancreas. 11. 14. 05. Both dogs still sick; the brown less prostrated than the white. 11. 15. 05. Both on feet; chloroformed, 11 A.M. Autopsies Brown (bile plus agar-agar). Peritoneum clear; no fat necrosis except spot or two just beside seat of operation. Corresponding to injected area of gland and extending slightly beyond, swelling and hæmorrhage of slight degree. No visible necrosis. The remainder of the organ appears normal. White (bile plus salt solution). Peritoneum normal except for fat necrosis in mesentery. Marked fat-necrosis about the pancreas. The mid-portion of the gland, except 2 cm. of splenic end, shows marked swelling, œdema, hæmorrhage, and fat necrosis.

The striking difference in the lesions caused, on the one hand, by bile of high colloidal quality, and, on the other, by the same bile in which the normal colloidal strength was reduced, is well brought out by this experiment. The protection afforded by colloidal substances may extend even further, so that the pancreas may entirely escape injury from a quantity of bile salts which, without such protection, is capable of causing severe lesions.

Experiment 10. 11. 17. 05. Two dogs of equal size. 11 – 2 A.M. Male: 2.5 c.c. bile + 2.5 c.c. 5 per cent. agar-agar. Duct not tied. Female: same mixture injected; duct tied. 11. 20. 05. Both dogs appear well. Female chloroformed. Peritoneum normal. No fat necrosis. Mid-portion of gland, receiving injection, moderately swollen and slightly œdematous, and on section slight hæmorrhage in tissue. No necrosis of parenchyma or fat. 11. 25. 05. Male chloroformed. The injection passed into the duodenal half and very little into the splenic parts of the gland. The peritoneum, excepting pancreas, normal. No fat necrosis anywhere. The injected end of the pancreas is white and glistening, the surface and section being granular. The granules include lobules and areas below lobules in size. The duct is patent. No evidence of hæmorrhage or necrosis.

It is clear from this experiment that by increasing sufficiently the colloidal strength of the mixture the bile can be made to act slightly upon, or be entirely prevented from causing acute lesions in, the pancreas. This experiment also suggests that, possibly, when the action is greatly diminished so that acute lesions fail to appear, the gland is yet sufficiently injured to respond with chronic productive lesions. Some years ago I showed[5]

[5] Flexner, *Johns Hopkins Hosp. Reports*, 1900, ix, 743; Flexner and Pearce, *Univ. Penna. Med. Bulletin*, 1901, xiv, 193.

that in the dog chronic pancreatitis, especially a focalized kind about acute lesions, easily develops. But the present experiment does not prove that the bile modified and reduced by agar-agar sets up alone the chronic lesions, for should the agar-agar not be easily eliminated from the ducts it would act as a foreign obstructive body and in this way itself cause chronic inflammation. Indeed, I have found that if a three-per-cent. suspension of agar-agar is injected into the duct in sufficient quantity it will cause, at the end of a week, a well-marked sclerosis. On section of the gland in these cases remains of the agar-agar can be found in the tissues.

That the biliary constituents, suspended in agar-agar, can pass out and come into relation with the gland tissue is shown to the naked eye by the loss of color which the agar suspension undergoes in the substance of the pancreas. This interchange of fluid between the gland and the agar suspension is a slow one relatively; and upon the rate of diffusion and the consequent degree of concentration of the biliary salts at any one time in contact with the parenchyma will depend, probably, the extent of the lesions.

Experiment 11. 11. 27. 05. Two dogs of equal size. 11–12 A.M. Brown: 2 c.c. bile plus 3 c.c. agar-agar. Injected duodenal half of gland. Black and white: 2 c.c. bile plus 6 c.c. agar-agar. Injected largest part of gland. 12. 4. 05. 11 A.M. Chloroformed. Brown dog: The injected portion of the gland is swollen and shows diffuse hyperæmia and punctiform hæmorrhages. The consistence is increased in this part, and the surface is granular. On section, the surface shows small mottled areas of hæmorrhage, fine yellowish, intra-acinar points of degeneration, and two larger softened areas of necrosis. The splenic half of the gland is kinked and at the angle an extraglandular cyst the size of a marble, containing clear fluid, has formed. The duct is patent until the bend is reached. It can be followed no farther. Beyond and about the cyst the gland is sclerosed. Black and white dog. Entire organ granular, sclerosed, and contracted. No acute lesions or fat necrosis. Main duct dilated slightly. In some parts the agar-agar has not been removed. The bile staining of the agar-agar has wholly disappeared.

This experiment calls for no discussion since it shows that with a sufficiency of colloid present the acutely injurious action of the bile can be prevented. It does not, however, settle the question of the relation of the chronic lesions to the diminished

action of bile salts since the agar-agar itself remained, in the one case, to act as a foreign body. Hence in the next experiment colloids were chosen which were free of the objection that they were capable of congealing in the ducts.

Experiment 12. 12. 2. 05. Two dogs of equal size. 12 M. to 1 P.M. Buff mongrel: Bile 2 c.c. plus mixture of mucin and nucleoproteid[6] 4 c.c. Injection entered duodenal half of gland. Hairy mongrel: Bile 2 c.c. plus 20 per cent. gelatine 4 c.c. Injection entered splenic half of gland. Ducts not tied. The first dog quickly recovered, the second never really recovered, from the operation. Chloroformed, 12. 9. 05. Autopsy: Buff dog: The entire duodenal half of the gland and a small part of the middle portion are swollen, white in color, and firm in consistence. The remainder of the splenic portion of the pancreas is normal in consistence and appearance except for a vivid congestion. The line of demarcation between the swollen and normal gland is sharp. No evidence of acute lesion and no fat necrosis exist. Duct not dilated. Hairy dog: At seat of operation fat necrosis in the pancreas. The splenic portion of the gland except the tip is swollen moderately and firm. The serosa of the tip contains small hæmorrhages, and the injected portion, on section, is the seat of marked necroses in the parenchyma and interstitial tissue. This part and the duodenal end are both somewhat sclerosed.

From these experiments the conclusion can be drawn that the suspension of bile in a bland mixture of high colloidal strength protects the pancreas from the immediate and acutely injurious action of the biliary salts. But besides this conclusion another can be drawn, namely, that a relation exists between the nature of the suspending colloid and the reaction excited in the pancreas. If, for example, the colloid is readily attacked by the pancreatic juice and the density of the mixture reduced, then the injurious salts may still be brought into such mediate relation with the gland as to produce moderately severe acute lesions; this would appear to be the case with gelatine. But with agar-agar and the mucin-nucleoproteid mixture, which are acted upon little if at all by the pancreatic secretion, the protection is so effective as to eliminate wholly gross injury to the gland tissues.

That this explanation is the correct one is further proved by the next experiment, in which gum arabic was employed as the diluent.

[6] A syrupy suspension of tendo-mucin and spleen nucleo-proteid was made in water by careful neutralization with $\frac{1}{10}$ N. sodium hydrate by Dr. Levene.

Experiment 13. 12. 14. '05. Two medium-sized dogs. 3.30 P.M. Hairy mongrel: Bile 2.5 c.c. plus gum-arabic suspension 2.5 c.c. Black terrier: Bile 2.5 c.c. plus saline 2.5 c.c. Ducts not tied. Both dogs up early next morning, but the first still very sick. About noon he refused to rise in his cage; dead, 4 P.M. Autopsy. The injection entered the duodenal half of the gland, which showed no marked inflammation, and no hæmorrhage or fat necrosis. There was slight swelling of the gland and a few necroses in the fat of the mesentery about the ducts. The cause of death of this dog is therefore not wholly clear The intestinal contents were blood-stained, and a few small broncho-pneumonic foci existed in the lungs. The terrier was chloroformed. Disseminated fat necrosis existed throughout the peritoneal cavity; it was very marked about the pancreas. The injection entered the mid-portion and splenic end of the gland. Fully two-thirds of the gland was the seat of marked acute inflammation associated with hæmorrhage. A blood clot of considerable size existed in the pancreatic and peripancreatic tissues about the middle of the gland.

This experiment is convincing in showing that by the addition of a bland colloid the action of bile on the pancreas can be greatly reduced or even entirely prevented; and it is also useful in showing that by reducing the colloid strength of normal bile by the addition of salt solution its injurious action can be considerably enhanced.

It would seem as if these conditions might be simulated in human pathology in instances in which the composition of the bile was modified by obstructive and inflammatory processes. The changes which imprisoned bile undergoes are well known. The loss of the diffusible salts and increase in muciginous constituents, which occur on obstruction of the ducts, and the accumulation of albuminous products, which takes place in inflammation, would provide exactly the theoretical conditions for the production of subacute or chronic lesions of the pancreas, rather than acute ones, provided this bile had been diverted into the pancreatic duct. If the conditions of these experimental lesions occur in human beings it seems not impossible that in this way may arise certain instances of partial or independent sclerosis of the pancreas which up to this time have received no adequate explanation.[7]

[7] Perhaps it may be worth while to indicate that a mechanism exists in nature, comparable with, if not the equivalent of, the syringe, for driving a colloidal suspension of considerable thickness into the pancreas. This mechanism is the

That biliary salts should act as poisons for pancreatic tissue and blood-vessels is, in view of the established cytolytic property of the salts for various kinds of cells[8]—blood-corpuscles, amœbæ, infusoria, muscle, nerve, ciliated epithelium, liver cells,—not remarkable. Since this power is so great and can be exerted against the liver itself some restraint would seem to be required in nature. This restraint, apparently, can be supplied by the colloids in and about the cells. It is, however, probable that all parenchymatous cells are not equally susceptible to injury by the bile salts, and the liver cells are stated by Rywosch to be especially resistant.

When one reflects upon the mechanism of the restraining action of colloidal substances upon bile salts, one naturally turns to the consideration of physico-chemical notions for an explanation of the effects observed.[9] Bile salts exhibit a low surface tension and hence diffuse readily. They tend therefore to pass quickly into a surrounding fluid medium; and in the case of the pancreas soon find themselves in contact with tissue cells and blood-vessels. The latter immediately respond to their presence by hyperæmia and extravasation; and the injurious action is soon extended to the pancreatic cells. The colloids, on the other hand, are composed of large molecules of high surface tension. When the two sets of substances are mixed in solution, the molecules of the salts are spread out over those of the colloid, whereby mechanical conditions producing decrease in diffusion velocity of the salts are brought about.[10] In order that severe injury may be inflicted upon the pancreas, a certain concentration of the salts must exist in the fluids in immediate relation with the tissues; and as these fluids are in process of constant change, this concentration must be reached in a unit of time. Any

gall-bladder and ducts, the muscular contraction of which might easily suffice for this purpose under conditions in which the biliary and pancreatic ducts had been, through pathological conditions, made continuous

[8] See Rywosch, *Inaugural Dissertation*. Dorpat, 1891.

[9] In view of the union of bile salts with proteids perhaps some account should be taken of this property.

[10] I am indebted to Professor Ostwald for this explanation. It was transmitted to me by the kindness of Dr. C. A. Herter.

marked reduction in diffusion velocity will, therefore, tend to reduce the action of the salts. Should the injury fall below a certain degree, and the action be prolonged, conditions favoring the production not of acute but of subacute or chronic lesions might be inaugurated. Moreover, the colloidal substances themselves while bland may not be without effect in causing new growth of connective tissue. For if the intrapancreatic pressure is too low to remove readily the colloid because of the higher friction which it exerts on the ducts, obstructive atrophy and sclerosis would result. But while this factor must be considered both in the experiments on dogs, and under similar hypothetical conditions in man, it can have no part in explaining the absence of the acute experimental lesions.

The rate of diffusion of crystalloids of low molecular weight into colloids (agar-agar) would appear, from Voightländer's [11] experiments, to be little lower than in water. Whether the same holds true for such large molecules as compose the bile salts I do not know; and I do not know, by direct experiment, whether a large molecular crystalloid, enclosed in a colloidal solution, would diffuse readily into a solution as complex as lymph. The experiments related in this paper would tend to speak against such ready diffusion. But it will be necessary to make the conditions of study simpler than those of these experiments before a final comparison of all the facts mentioned can be made.[12]

[11] *Zeitsch. f. physik. Chemie*, 1889, iii, 316.

[12] Experiments relating to these questions are being pursued.

ON THE CARBOHYDRATE GROUP IN THE NUCLEOPROTEID OF THE SPLEEN.

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(From the Rockefeller Institute for Medical Research, New York.)

By the term nucleoproteids is designated that class of substances which on cleavage yield, besides the usual components of the proteid molecule, phosphoric acid, purin bases, pyrimidin derivatives, and carbohydrates. While in recent years much progress has been made in our knowledge of the basic constituents of nucleoproteid, our knowledge of the carbohydrate moiety of the molecule has remained very imperfect. The present note deals with the results of our further investigation of the proteid derived from the spleen; but it is our intention to study, in a similar manner to that mentioned here, the proteids from other organs. We have succeeded in obtaining from the spleen a substance presenting the properties of glucothionic acid—that is, a nitrogenous body composed of sulphuric acid and carbohydrate.

Hitherto substances of this class had been obtained by Moerner and by Schmiedeberg from cartilage, by Krawkaw and Odi from amyloid, by one of us (Levene) and Gies and his co-workers from mucoids and mucin. Levene had also shown it to exist in the parenchymatous organs, a fact later extended by the present writers.

In the present study the nucleoproteid of the spleen was prepared in the usual manner by boiling the organs with water and acidulating the extract with acetic acid. The precipitate was washed by decantation and extracted with alcohol or with alcohol and ether. The air-dry product contained two per cent. of phosphorus. This proteid was subjected to the treatment already described by Levene [1] for obtaining glucothionic acid.

[1] *Zeitsch. f. physiol. Chem.*, 1903, xxxvii, 400.

By this means a substance has been obtained which, while not absolutely free from nucleic acid, yet contains (one sample tested) 8.45 per cent. of nitrogen and 3.19 per cent. of sulphur. This body reduces Fehling's solution only after previous hydrolysis with mineral acids, and gives a pronounced reaction for pentose with orcin-hydrochloric acid. On cleavage of the proteid itself with dilute mineral acids a substance presenting the same properties was obtained. The proportion of sulphuric acid was, however, much lower.

Two interpretations of this finding occur to us. First, substances commonly designated nucleoproteid are composed of two distinct substances—mucoid and true nucleoproteid. Second, they contain in their molecule a glucothionic acid. We are at present engaged upon a further study of this problem.

ON A CERTAIN CRYSTALLINE BODY OBTAINED ON THE PROLONGED DIGESTION OF GELATINE.

By P. A. LEVENE, New York.

(From the Rockefeller Institute for Medical Research, New York.)

In course of a study of the products of tryptic digestion of gelatine the writer demonstrated the formation of prolin.[1] While preparing a large quantity of prolin recently a crystalline substance was obtained which differed in its properties from the body sought. This substance is soluble in absolute alcohol and readily crystallizes from its solution in this solvent. It is precipitated by phosphotungstic acid, but is not precipitated by iodide bismuth-iodide potassium. Thus far an insoluble platinum salt has not been obtained; nor has a salt been formed with copper oxide. A picrate was prepared by means of an alcoholic solution of picric acid. The substance tastes strongly bitter and gives a pronounced test for pyrrol. The melting point of the pure substance is 182°–185° C., and of the picrate, 165°–167° C. The elementary composition is as follows: C = 53.98 %; H = 6.67 %; N = 18.51 %; O = 20.84 %. The calculated formula is $C_7 H_{11} N_2 O_2$. C = 54.19; H = 7.09; N = 18.09; O = 20.63 %. The formula calculated for $C_7 H_{10} N_2 O_2$ is C = 54.55; H = 6.49; N = 18.18; O = 20.78 %. A nitrogen estimation of the picrate gave 18.58 %. As calculated for $C_7 H_{10} N_2 O_2 . C_6 H_2 (NO_2) OH$ the nitrogen is 18.27 % and as calculated for $C_7 H_{10} N_2 O_2 . 2C_6 H_2 (NO_2)_3 OH$ it is 18.30 %.

Hence it is probable that the composition of the substance is $C_7 H_{10} N_2 O_2$, and that it is in some way related to prolin. Further investigation into the nature of this body is in progress.

[1] *Zeitsch. f. physiol. Chem.*, 1903, xli, 99.

GASTRIC ULCERS IN RABBITS FOLLOWING RESECTION OF THE PNEUMOGASTRIC NERVES BELOW THE DIAPHRAGM.

By W. OPHÜLS, M.D.

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PLATE XI.

The experiments reported in this paper were suggested by an article published by Van Yzeren,[1] one of Talma's assistants, in 1901. Van Yzeren cut both vagi in twenty rabbits below the diaphragm and observed the development in ten of chronic ulcers, usually single, in the pyloric region of the stomach. His results appear more remarkable still when we take into account that of the ten rabbits without ulcers, seven lived five days and less (five only one day), whereas the first ulcer was found in a rabbit that had lived seven days. Of the rabbits examined later, three only did not show ulcers (one examined on the ninth, one on the tenth, and one on the forty-seventh day). The last rabbit was examined 289 days after the operation and showed an ulcer still present.

Van Yzeren was preceded in this work by two Italian investigators: Lorenzi and Saitta. In 1893, Lorenzi[2] reported that after resection of the vagi either at the neck or below the diaphragm in rabbits, hemorrhages were frequently observed in the mucous membrane of the stomach and in some cases from these, when the animals survived longer than twenty-four hours, hemorrhagic erosions developed. In 1900, Saitta[3] performed

[1] Van Yzeren, "Die Pathogenesis des chronischen Magengeschwürs," *Zeitsch. f. klin. Med.*, 1901, xliii, 181.

[2] Lorenzi, "L'influenza del sistema nervoso nella patogenesi dell' ulcera dello stomaco," *Rassegna di Sc. med.*, 1893.

[3] Saitta, "Contributo alla patogenesi dell' ulcera gastrica," *Gaz. degli Ospedali Milano*, 1900, xxi, 599.

bilateral vagotomy at the neck in sixteen rabbits, in which he treated as well as possible the resulting disturbances in respiration and heart action. Three of the rabbits died on the second day and showed normal stomachs, three died on the eighth to the tenth day and showed black ecchymotic spots in the mucosa, and the remaining four died on the fifteenth to the twentieth day, showing hemorrhages and erosions in the mucous membrane. In four rabbits that had been operated upon in a similar manner and had been given 3 per cent. hydrochloric acid solution by mouth, he found multiple ulcers.

In 1901, dalla Vedova [4] studied the influence of disturbances in innervation on the gastric mucous membrane of dogs. His experiments were not confined to the vagus nerve, but included the ganglia cœliaca and the left splanchnicus magnus. The nerves were either resected, or alcohol, which, according to him, has the same effect, was injected into them. In six instances he resected the dorsal branch of the vagus below the diaphragm or injected alcohol into it without result. In twelve animals he succeeded in removing the left or both ganglia cœliaca. Positive results were observed in five of the latter. He removed the left splanchnicus magnus successfully in ten dogs, with nine positive results. These animals lived from one to sixty days. In all the positive cases but two he found hemorrhages and hemorrhagic erosions, and in the two remaining he found larger hemorrhagic, funnel-shaped ulcers. The last occurred after resection of the ganglion cœliacum, the animal living three days, and after injection of alcohol into the left splanchnicus magnus, the dog surviving the operation eighteen days.

The most recent paper on the subject, which is quite full, was published by Donati.[5] Donati resected both vagi below the diaphragm in nine dogs and eleven rabbits, extirpated the plexus cœliacus in eleven dogs and five rabbits, and removed both of these at the same time in one dog. He also resected

[4] dalla Vedova, "Ricerche spirimentale sulla patogenesi dell' ulcera gastrica," *Arch. ed atti della soc. ital. di chir.*, 1901; *Archiv für Verdauungskrankh.*, 1902.

[5] *Archiv für klin. Chirurgie*, 1904, lxxiii, 908.

A short notice of the same work also appeared in the *Zentralblatt für Chirurgie*, 1904, xxxi, 346.

both vagi at the neck in six dogs, but these animals lived only four to five days. He resected one vagus at the neck in three dogs, the animals being killed between the thirtieth and sixty-fifth day; and in another dog he resected one vagus at the neck and later the other one, the animal surviving eighty-one days. In all these experiments the results were entirely negative. Donati gives a very complete review of the literature, inclusive of the experiments made by physiologists in order to ascertain the function of the gastric nerves, and points out that as far as lesions in the mucous membrane of the stomach are concerned the results have been most contradictory. From his review of the literature and from the result of his own experiments he comes to the conclusion that very probably the hemorrhages, erosions, and even ulcers, described by other observers, were entirely accidental, and that disturbances in innervation have no effect whatever upon the mucous membrane of the stomach.

In spite of Donati's work it seems difficult to understand how the results of Van Yzeren could be due entirely to accident. Even granted that hemorrhages and erosions are comparatively frequently found accidentally in the gastric mucosa of animals, especially in rabbits, and my own experience leads me to be certain of this, still typical chronic ulcers have, at least as far as I am aware, never been described as occurring spontaneously in these animals. This point seems all the more important as rabbits are so very commonly used for experimental purposes and on that account carefully examined. In the opinion that chronic ulcers of the stomach do not occur spontaneously in rabbits I am confirmed by observations made on a considerable number of controls in the course of my own experiments on this subject.

In these experiments I have confined myself entirely to rabbits and have always resected both vagi below the diaphragm. The operation is easily performed. The animals are anæsthetized with ether, an incision is made along the free margin of the ribs on the left side, and the stomach is exposed and drawn downwards. After tearing through a thin peritoneal fold, which runs from the stomach to one of the lobes of the liver, the lower end

of the œsophagus, which passes quite a distance through the diaphragm, is exposed; the nerves can then be easily found and a piece of about ½ cm. in length resected without producing any hemorrhage to speak of. If the anæsthetic is carefully given the animals recover very rapidly from the operation, and with fairly careful antiseptic precautions one should not lose any of them by accident. The animals operated on were examined at varying intervals. They do not as a rule show any bad symptoms as a result of the operation and remain in a fairly good state of nutrition. In a few instances only was a considerable degree of emaciation noticed, although not necessarily in those animals in which gastric ulcers developed. A careful examination of the site of operation was made in all cases in order to determine whether both vagi had been cut and, also, in order to exclude any complications resulting from a possible infection at the point of operation. In the first days following the operation one notices very little reaction. Later, a small amount of dense cicatricial tissue develops in which the cut ends of the vagi are embedded and which, on the other side, adheres to the cardiac end of the lesser curvature of the stomach. The amount of cicatricial tissue found was no greater in the animals developing ulcers than in the ones which gave negative results. I have never been able to find any macroscopic evidence of regeneration of the nerves.

Thirty rabbits were operated upon in this way and the results are shown in Table I, page 185. The transverse fold mentioned is one which is plainly seen when one examines *in situ* the interior of the stomach of a rabbit through an incision along the greater curvature. Several smaller and one large transverse fold are then noticed at the lesser curvature between cardia and pylorus and a little nearer to the pylorus. This fold becomes narrower and lower on both sides and disappears before it has reached the greater curvature.

There were then six positive results in thirty experiments, or, if we exclude those animals examined before the twenty-fourth day, at which day the first ulcer was found, six positive results in eighteen experiments were obtained. It is at once apparent

that in my series the ulcers developed much less regularly than in Van Yzeren's series. Whenever they were found, however, they corresponded very closely to Van Yzeren's description. They were undoubtedly chronic ulcers, as was shown by the marked induration at the base; they appeared of round or oval form, with sharp edges and smooth bottom. All the ulcers which I ob-

TABLE I.

No. of Rabbits Examined.	Day after Operation.	Result.
1	3d	Negative.
2	5th	"
2	7th	"
1	8th	"
1	10th	"
1	11th	"
2	15th	"
1	17th	"
2	18th	"
1	24th	Transverse oval chronic ulcer about 8 x 10 mm. on summit of fold. Sharp edges, smooth bottom.
1	26th	Negative.
1	34th	Small chronic ulcer on fold.
1	39th	Negative.
1	42d	"
1	45th	"
3	50th	In one, small chronic ulcer 2 mm. from pylorus.
1	60th	Chronic oval ulcer 7 x 9 mm. on summit of fold; sharp edges.
2	64th	In one, nearly healed ulcer with much induration on summit of fold.
4	71st	Negative.
1	88th	Small chronic ulcer on anterior wall in pyloric region, but near fundus.

NOTE.—Of thirty rabbits operated upon and examined six showed ulcers of the stomach. No ulcer found earlier than 24th day. Eighteen rabbits examined after 24th day; hence ulcers developed in 33 per cent. of these rabbits.

tained were single, while Van Yzeren observed several instances in which they were multiple. Since Van Yzeren neglected to give any pictures of the ulcers I have photographed one of the larger ones, which is shown in natural size in Fig. 1. This photograph also shows well the relation of the ulcer to the transverse fold. Sections were made of two of the larger ulcers. The ulceration extended, in one case, into the muscle, and in the

other, of which a microphotograph is given (Fig. 2), through the muscle into the subperitoneal tissue. In both ulcers there was a considerable development of cicatricial tissue at the base, which was covered with a thin layer of necrotic material including fibrin, blood, remnants of food, etc. The mucous membrane at the edge, as may be seen in the microphotograph, may show a considerable hypertrophy and project over the ulcer. The larger arteries and veins near the ulcer were carefully examined in numerous sections; they were found to be entirely normal.

During the course of the experiments I have examined the stomachs of a large number of normal rabbits. I have several times found small hemorrhages, and in one case I found a hemorrhagic erosion with complete destruction of the mucous membrane over an area of about 2 mm. in the center, on the summit of the above described fold, but the process was evidently quite recent. At the bottom of the defect there was a narrow zone of necrosis and a few leucocytes could be seen in the tissues beneath, but no other reaction was visible. I have examined certainly as many normal rabbits as operated ones. All our rabbits are kept together in a large rabbit pen, to which the animals operated on were returned after they had sufficiently recovered from the operation. Hence all the rabbits eat the same food and are otherwise kept under identical conditions. The rabbits used in the experiments were taken from the same lots as were those which were examined without operation.

It could therefore hardly be a matter of chance that the chronic ulcers were found only in the animals operated upon which survived the operation twenty-four days or longer. I have already mentioned that if chronic ulcers in rabbits occur at all spontaneously, they must be very rare indeed. I believe, therefore, that in spite of Donati's eleven negative experiments we are safe in concluding that resections of both vagi below the diaphragm in rabbits may be followed by the formation of peculiar chronic ulcers beginning in the mucous membrane of the stomach, but that this result is not either constant or remarkably frequent.

The question arises as to what relation exists between the

section of the nerves and the production of the ulcers. We should enquire, naturally, first of all, of the function which the vagus nerve exerts upon the stomach and what the disturbances of this viscus are after the nerve is cut.

It can hardly be doubted that the motions of the stomach are to a certain extent under the influence of the vagus nerve. That this is true can be plainly seen upon stimulating electrically or otherwise the two vagi below the diaphragm. The result is a strong contraction of the muscle of the stomach, which seems to be similar to the normal contractions, but more vigorous; and, if the stimulation is not so strong as to produce a tonic contraction, the contractions are repeated more frequently. It is also claimed by some that the vagus nerve contains inhibitory fibres. If, on the other hand, the nerve is cut, it has been found that the movements of the stomach are retarded, but that otherwise they continue unchanged. In my experiments I noticed in the first weeks a marked dilatation of the stomach. The normal capacity of an ordinary rabbit's stomach is about 100 to 120 c.c. The capacity was increased often to 180 c.c. or more. At the same time in these instances it was noticeable that the elastic properties of the gastric wall were unaltered. With the increase in contents there was a marked rise in pressure within. The dilated stomachs therefore felt very hard to the touch, and on that account could be easily palpated through the abdominal wall in the living animal. The operated animals seem somehow to overload their stomachs. I believe that it is this condition that misled Van Yzeren and led him to assume that tonic cramps of the muscle occurred after the resection; at least I have never seen any evidence of the occurrence of such cramps. In all my instances the hardness of the organ was plainly accounted for by overfilling. This disturbance of motion seems in course of time to be compensated, for in all the animals examined late the stomach had returned to its normal size and the tension of the wall had become normal.

On the whole, the vagus seems to have very little influence upon the secretion of gastric juice. Pawlow, it is true, has demonstrated that the central reflex for the secretion of the gastric juice

passes through the vagus, because in his dogs the secretion of gastric juice ordinarily taking place when they see tempting food is missing when the vagus is cut. But our rabbits seem to have done well in the absence of this central reflex. Direct stimulation of the nerves below the diaphragm is followed by the appearance of considerable gastric juice upon the mucous membrane, which phenomenon can be repeated several times after the secretion has been wiped away. Still, it is of course impossible to decide whether an actual secretion takes place as a result of the stimulation or whether preformed gastric juice is simply squeezed out of the mucous membrane by the contraction of the muscular elements in it. In any case the proper secretion of gastric juice does not seem to have been disturbed materially in our rabbits by the section of the vagi. The contents of the stomach of the operated animals had absolutely normal appearances and the chemical examination did not reveal any constant changes. The result of the chemical examinations made by Töpfer's method in several rabbits is given in Table II. The figures represent the number of cubic centimeters of decinormal solution of sodium hydroxide necessary to neutralize 100 c.c. of gastric juice. The latter was obtained by adding an equal volume of distilled water to the gastric contents and filtering. The figures obtained with the filtrate were doubled. The method may not be entirely accurate, but it gives comparable values. It was of course impossible to obtain the gastric juice always at the same stage of digestion, which probably accounts for the rather wide discrepancies in the figures.

Another possibility that suggests itself as an explanation of the formation of the ulcers after resection of the vagi is the disturbance of those protective mechanisms which seem to exist in the gastric and intestinal mucous membranes in order to prevent undue injury to them from their contents. These mechanisms have been described by Exner [6] and by Müller.[7] These

[6] Exner, "Wie schützt sich der Verdauungstract vor Verletzungen," *Arch. f. Phys.*, 1902, lxxxix, 253.

[7] Müller, "Beitrag zur Kenntnis von den Schutzeinrichtungen des Darmtractes," etc., *Arch. f. Phys.*, 1904, cii, 206.

authors found that mechanical irritation of the mucous membrane of the intestines and of the stomach in dogs and cats produces, first, an anæmia at the point of irritation, which is followed by a small dimple resulting from the contraction of the muscularis mucosa at the spot. Exner had already noticed that the same phenomenon occurs in the intestines of animals shortly after death, or even when pieces of intestine have been cut out. Müller sometimes observed a disappearance of the phenomenon in the stomach of cats after resection of the vagi below the diaphragm, but at other times there was no change.[8]

TABLE II.

	Total Acidity.	Free HCl.	Loosely Comb. HCl.	Organic Acids and Acid Salts.
Normal rabbit.....	124	96	4	24
" "	88	76	8	42
" "	148	128	16	4
" "	132	132		
Rabbit 5 days after operation.....	92	72	4	16
" 11 " " "	96	48	16	32
" 42 " " "	100	80		20
" 50 " " "	112	72	8	32
" 50 " " "	132	104	16	12
" 50 " " " with small ulcer in pyloric region.....	88	48	8	32

I was led to make similar experiments upon the mucous membrane of the stomach of rabbits by the fact that the disturbances arising from section of the vagus at the neck are commonly ascribed partly to interference with normal reflexes, such as, for instance, the laryngeal reflex. The gastric mucous membrane of rabbits also responds to mechanical irritation in a definite and similar manner to that described for cats and dogs by

[8] Both Exner and Müller describe these phenomena as due to reflex action. I have purposely avoided this term in the above description, because in spite of their arguments to the contrary it does not seem definitely settled whether they could not be the result of direct stimulation of the muscular elements in the mucous membrane. It is probably the chief if not the only function of the muscularis mucosæ to draw the mucous membrane away from points of mechanical irritation.

Exner and Müller. It is interesting to note that the response of the mucous membrane is much more prompt and marked in the region of the lesser curvature, which is the usual site of the ulcers. This difference is so distinct, that while in the fundus one notices hardly any effect on pricking or touching the mucous membrane with a blunt instrument, the anæmia and the motion in the mucous membrane are quite evident under similar conditions at the lesser curvature. As a result of the first tests in which I irritated the mucous membrane chemically, a form of irritation to which it reacts much less promptly, I had the impression that cutting of the vagus interfered with or completely abolished the reaction; but this opinion has not been borne out by my later experiments with mechanical irritation. Indeed it would seem as if the reaction was sometimes even more marked after the vagi were cut. The reaction also persists for a time after section of the vagi. Animals were examined at varying intervals after operation and no change in the reaction was noticed. In one case I had the opportunity of testing for the reaction in a stomach that was the seat of a chronic ulcer. No reaction could be elicited in the immediate neighborhood of the ulcer, but this was probably due to the immobilization of the mucous membrane by the chronic inflammatory process. A little farther away the reaction was weak, but undoubtedly present. It cannot therefore very well be any interference with this protective reaction that causes the ulcerations.

I believe that originally the ulcers are traumatic in origin, due to the presence of hard, more or less pointed, substances in the food. This explanation would account for their occasional or more frequent presence, depending on accidental circumstances, and serve to harmonize my results with those of Van Yzeren. Their position on the summit of a projecting fold is suggestive also of this mode of origin. The hemorrhages and erosions observed in the stomach of normal rabbits are also probably due to the same cause. It seems, however, very difficult to explain why the ordinary defects produced in this way heal rapidly, while after section of the vagi a tendency to development of chronic ulcers exists.

Van Yzeren bases his explanation of the origin of the ulcers on the occurrence of cramps in the muscle, which he claims to have observed. He believes that the cramps interfere with the blood supply of the mucous membrane and produce an anæmic necrosis at the point where the ulcer is to develop. He found confirmation of this theory in certain experiments in which he not only cut the vagi, but also made deep incisions into the muscle from the outside, or performed gastroenterostomy. If the latter operations, designed to relieve the cramps, were added, no ulcers developed. Quite apart from the fact that it seems more than doubtful whether such cramps actually occur, it is not easy to understand how they could produce a localized anæmic necrosis; and, even assuming that they do so, it still remains to be explained how such an obstacle to the usual rapid healing of defects could operate. Since the ulcers do not occur as regularly as Van Yzeren believed, his comparatively few experiments designed to prevent the occurrence of them by the above described means are, to my mind, hardly conclusive.

One could imagine that traumatism is more apt to occur when the stomach is overfilled and very tense, as is the case in the first week after vagotomy; and that the increased pressure of the contents might also stand in the way of a proper healing of any defect produced in this fashion. Unfortunately, for this supposition, the motor conditions seem to return to the normal after a little while. Still, it is a well-known fact that after chronic ulcerative processes have once been started, healing may be retarded though the pathological conditions that caused the ulceration be removed.

It seems necessary to discuss one other possibility, namely, that the ulcers belong to the so-called tropho-neurotic lesions; that is, that through the resection of the nerves trophic influences are removed which are necessary to preserve the normal resistance of the mucous membrane. I believe this explanation should be accepted only after all other possible explanations have been exhausted, and yet from the data on hand, it has certainly to be considered. There is something mysterious about the assumption of such tropho-neurotic influences that is

somewhat contrary to our ordinary modes of reasoning; and still it cannot be denied that certain well-known facts could hardly be explained otherwise than on the basis of some such hypothesis.

I believe then that my experiments have shown that in rabbits there is some relation between the resection of the vagi below the diaphragm and the occurrence of chronic ulcers of the stomach. The experiments, I am sorry to say, have not shed much further light on the question of what this relation is. I also believe that it would be entirely premature to make use of these experiments in the explanation of peptic ulcer in man, although I acknowledge that the resemblance between the two processes is striking.

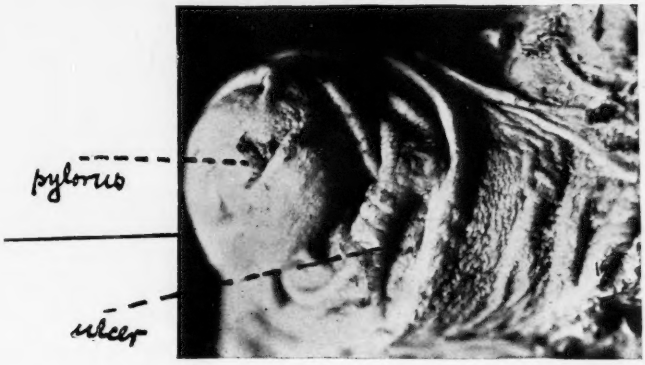

FIG. 1

FIG. 2